

SOME STUDIES IN FOREST AND
AGRICULTURAL MYCOLOGY

A thesis presented to the
Faculty of Science

of the

University of Edinburgh

by

ANTONY TRAFFORD KERNOT CORKE

for the degree of
Doctor of Philosophy

October 1955.



The thesis is presented in two parts.

PART I.

A study of Cucurbitaria piceae Borthwick,
a disease of the buds of the Spruce.

PART II.

A study of Pseudopeziza ribis Kleb. (Gloeosporium
ribis (Lib.) Mont. and Desm.), the Leaf Spot disease of
the black currant.

The work which forms the basis for Part I was carried
out at the Department of Botany of the University of
Edinburgh prior to September 1952. It was continued,
under leave of absence, together with the work described
in Part II, at the Department of Agriculture and Horticul-
ture of the University of Bristol.

PART I.

A study of CUCURBITARIA PICEAE

BORTHWICK.

Introduction	25
History of the study	26
<u>Locus of infection.</u>	27
<u>Method of infection.</u>	

CONTENTS.

	Page.
<u>INTRODUCTION.</u>	
<u>CLASSIFICATION.</u>	2
<u>DISTRIBUTION.</u>	
<u>EXPERIMENTAL.</u>	4
<u>PRESERVED MATERIAL AVAILABLE FOR STUDY.</u>	
<u>MATERIAL AVAILABLE FOR FIELD STUDY.</u>	
<u>EXAMINATION OF FRUCTIFICATIONS.</u>	5
<u>Perithecia.</u>	
<u>Pycnidia.</u>	8
<u>Pycnospores from other sources.</u>	9
<u>Spore discharge and germination.</u>	11
<u>GROWTH IN CULTURE.</u>	14
<u>Media.</u>	
<u>Rate of Growth.</u>	
<u>Colour.</u>	16
<u>Special media.</u>	18
<u>MISCELLANEOUS LABORATORY EXPERIMENTS.</u>	19
<u>SOME ASPECTS OF THE HOST/PARASITE RELATIONSHIP.</u> ..	20
<u>Locus of infection.</u>	21
<u>Method of infection.</u>	

	Page.
<u>Depth of penetration.</u>	22
<u>Nutrition.</u>	
<u>Extent of damage to buds.</u>	23
<u>Damage to shoots.</u>	26
<u>Formation of axillary buds.</u>	27
<u>Effect on growth of the host.</u>	28
<u>FIELD AND LABORATORY OBSERVATIONS.</u>	29
<u>INFECTION EXPERIMENTS.</u>	31
<u>THE EFFECT OF CLIMATE ON THE LIFE-HISTORY.</u>	33
 <u>DISCUSSION.</u>	 36
 <u>SUMMARY.</u>	 42
 <u>REFERENCES.</u>	 44

INTRODUCTION.

In 1906 Dr. A.W. Borthwick of the University of Edinburgh noticed a fungal disease of the buds of an ornamental spruce. The host was a young specimen of Picea pungens var glauca on an estate at Abercairney in Perthshire, Scotland.

Examination of diseased buds showed them to be densely covered with the black, spherular pyrenocarps of a fungus, a new species of the genus Cucurbitaria. The disease was subsequently described and the fungus named Cucurbitaria piceae Borthwick (3).

Descriptions of this disease have since been published by Ferdinand'sen and Jørgensen (6) and Müller (15). The remainder of the literature concerning this fungus records its discovery in new localities and shows it to be extremely sporadic and, so far as is known, confined to Europe. In the British Isles the fungus has been observed only in Scotland where the usual hosts have been Picea pungens and its variety glauca, and Picea excelsa.

It is fortunate that the attacks by this fungus have so far been limited to small numbers of trees in scattered areas, since the latter species is widely planted as a timber tree in this country, whereas Picea pungens and its variety glauca is planted solely for decorative purposes. On the Continent of Europe,

however, the situation is very different. There the disease has reached epidemic proportions in some areas where both these species are planted in large numbers; they have been heavily attacked not only as mature and young trees in forest plantations, but as seedlings in forest nurseries. For this reason there is considerable danger of attacks by this fungus causing widespread damage in our own forestry plantations, and a solution to the problem of its control is desirable.

C L A S S I F I C A T I O N

Cucurbitaria Berberidis Persoon, a well-defined and widely distributed species on Berberis L., is considered the type of the genus. The generic name is due to Gray (9), but subsequent study of the genus as described by him, led to the removal of three of the four original species. The family Cucurbitariaceae was first described by Lindau (13), after a number of attempts had been made to describe the characters limiting the genus Cucurbitaria, and to classify it correctly. Recently a monograph relating to those species of the genus found in North America (22), has done much to define and to establish the true limits of the genus, although its position in the scheme of classification has long been firmly established in the Sphaeriaceae Dictyosporae by the character of the ascospore (18).

D I S T R I B U T I O N

Although Cucurbitaria piceae has mostly been reported on Picea pungens Engelmann, Picea pungens var. glauca Regel and Picea excelsa Link, it has also been found in single instances on Picea sitchensis Trautvetter and Meyer and Abies pectinata De Candolle (4). In addition to this latitude in the choice of a host, the fungus has shown variation in the character of its attack apparently unrelated to the age or species of its victim.

In the scattered appearances of this disease on Picea pungens in Scotland the damage has been slight and confined to the lower branches of young trees. In N. Bohemia on the other hand, in forests at Bad Königswart, the disease has been widespread on this species and has caused severe damage (20). With regard to attacks on Picea excelsa, those in Scotland have again been light but in this case, as in Denmark, on the upper branches of mature trees (6). The damage done in Denmark, however, has been severe in some areas; 25 per cent of the trees in a sixty-year-old plantation at Brørup in S.W. Jutland were so heavily infected that growth was practically arrested. In contrast, only single trees contracted the disease on the island of Bornholm and in North Zealand.

It is of interest to note that Picea excelsa, one of the species most commonly attacked, is of European origin whereas the other, Picea pungens is a native of Western North America where this disease is unknown (22), and it is perhaps significant that the reports of the appearance of this fungus on new species (23, 6) should be amongst the most recent.

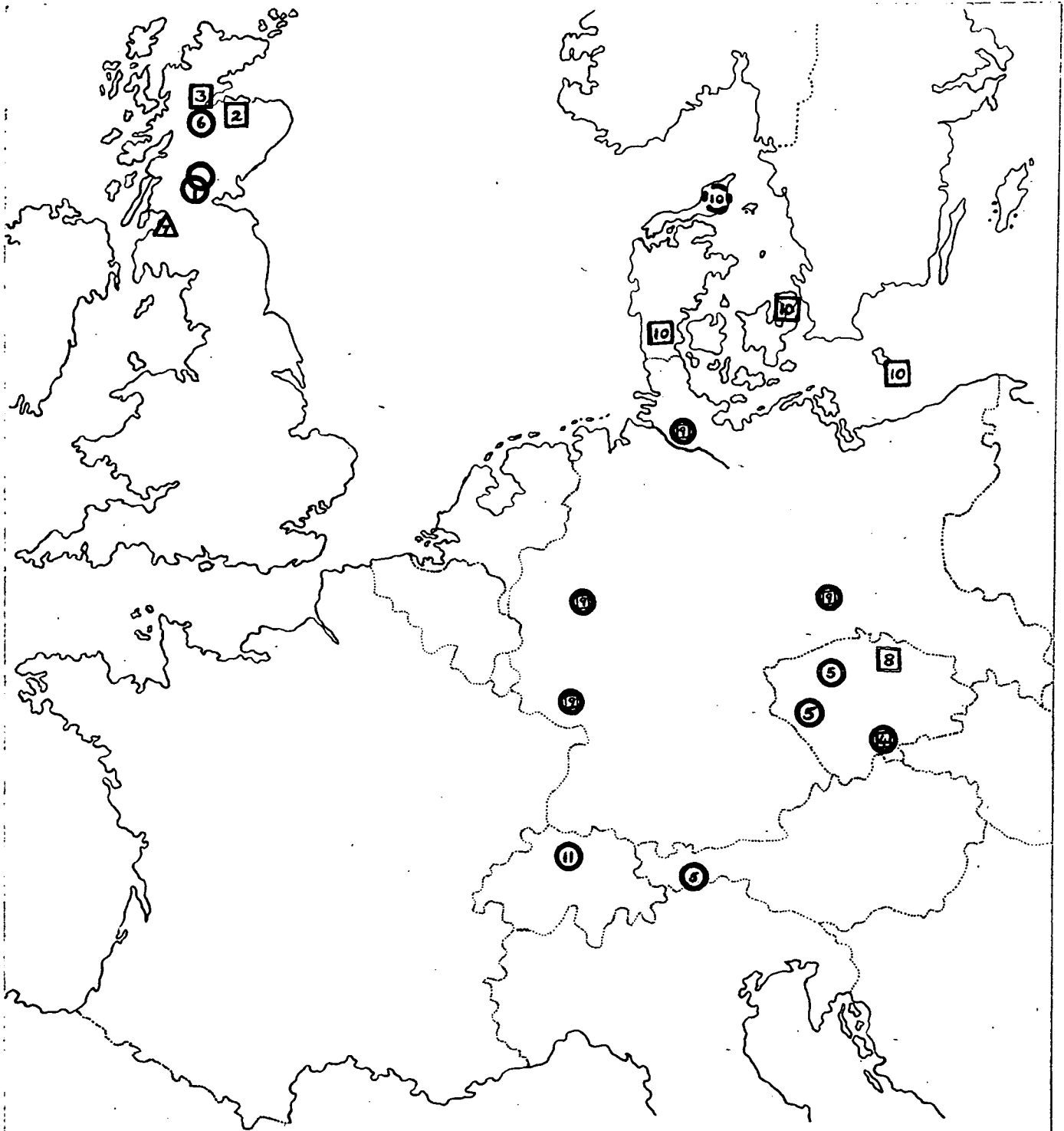
Text-figure 1 shows the present known geographical distribution of Cucurbitaria piceae Borthwick. The host species in each case is represented by a symbol. The numerals indicate the order in which reports concerning the disease appear in the literature, to which reference is also made.

TEXT-FIGURE I.

Distribution map of Cucurbitaria piceae Borthwick.

- 1. 1909. Abercairney, Perthshire, Scotland. (3).
- 2. 1917. Darnaway Forest, Near Forres, Morayshire, Scotland. (21).
- 3. 1918. Beauly, Inverness-shire, Scotland.
- ◼ 4. 1918. Kaiserwald-Gratzen (Gratzen or Navé Hradý), S. Bohemia. (12).
- 5. 1919. Karlsbad (Karl Vary), N. Bohemia. (20).
- Bad Kőnigswart (Kinzwart), N. Bohemia. (20).
- Madonna di Campiglio, S. Tyrol. (20).
- 6. 1924. Dochfour, Inverness-shire, Scotland.
- △ 7. 1932. Benmore Estate, Nr. Kilmun, Argyllshire, Scotland. (23).
- 8. 1937. Reichenberg (Liberec), N. Bohemia. (20).
- ◼ 9. 1938. Forest Nurseries (e.g. Halstenbek - Rellingen,
 Rinnsecke, Knittelsheim, Liebenwerda), Germany. (14).
- ◉ 10. 1938. Østerild Plantation (Hammer Hill Nr. N. Sundby), Thyland,
 Denmark. (6).
- Stilde Plantation, Nr. Brørup, S.W. Jylland, Jutland,
 Denmark. (6).
- Rude and Grib Forests, N. Sjaelland (Zealand),
 Denmark. (6).
- Bodilsker and Klemensker Plantations, Bornholm,
 Denmark. (6).
- 1946. Dunkeld, Perthshire, Scotland.
- 11. 1950. Kősnőcht, Itschnach Kt. Zurich, Switzerland. (15).

Dr. Mőller in private correspondence dated September 29th 1954 reported the recent discovery of C. piceae on Picea excelsa at Arosa, Kt. Graubőnden, Switzerland.



LEGEND

○ *Picea pungens*
◉ *Abies pectinata*

□ *Picea excelsa*
Δ *Picea sitchensis*

E X P E R I M E N T A L

PRESERVED MATERIAL AVAILABLE FOR STUDY.

- a) Type material gathered by Dr. Borthwick from Picea pungens var glauca at Abercairney, Perthshire in May 1906 and kept in water until August before being dried (3). (Herb. Hort. Edin.).
- b) An infected bud of Picea excelsa from Darnaway Forest, Morayshire.
(Herb. M. Wilson).
- c) An infected bud of Picea excelsa from Beaully, Inverness-shire.
(Herb. M. Wilson).
- d) Infected branches of Picea pungens collected at Dochfour, Inverness-shire by Dr. Borthwick in June 1924 and preserved in spirit.
- e) Infected branches of Picea pungens collected at Dunkeld, Perthshire by Dr. M. Wilson in April, and preserved in spirit.

In addition to the preserved material which was available only for limited microscopic examination, fresh material was obtained.

- f) Infected twigs of Picea pungens var glauca from Küssnacht, Itznach, Kt. Zürich, Switzerland collected by Dr. E. Müller of Zürich.
- g) Infected branches of Picea pungens collected at Dunkeld, Perthshire between September 1950 and August 1952.

MATERIAL AVAILABLE FOR FIELD STUDY.

On a private estate at Dunkeld in Perthshire, Scotland, a small group of Picea pungens stands almost at the foot of the eastern side of the Tay valley. To the east the steeply rising ground is densely planted with deciduous and coniferous trees, while to the west the open ground falls away for a short distance to the river bank.

The trees, which were about fifteen years old in 1951 appear to be moderately healthy; closer inspection, however, reveals the presence of a large number of damaged buds, particularly on two trees

of the group. Here, most of the infected buds are to be found where the closely interwoven lower branches of adjacent trees afford some protection from the drying effects of sun and wind.

Collections of material from this source were made to a height of about eight feet to include as much variety as possible in the type and severity of the infection.

Also available for experimental work were three young Picea pungens at the Department of Botany, University of Edinburgh.

EXAMINATION OF FRUCTIFICATIONS.

Perithecia.

The fructifications arise as small globular masses of hyphae at the point where the pseudoparenchyma meets the outermost bud-scale. The pressure exerted by the growing mass eventually causes the cells of the bud-scale to rupture and expose the surface of the fructification. The outermost cells, which have meanwhile become heavily pigmented, become black on exposure, a process which continues throughout the development of the fructification.

A number of perithecia were found to be about $445\ \mu$ wide and $510\ \mu$ in length, the latter measurement being that of the body of the perithecium without the stalk. The sporogenous cavity was found to be about $330\ \mu$ in length and $325\ \mu$ wide (Text-fig. 2).

The perithecial wall, or peridium, is composed of three layers of cells having different characteristics. The outermost layer of the wall is composed of heavily pigmented thick-walled cells, two or three in depth, and with a total thickness of $19-23\ \mu$. The cells are more or less spherical, $9\ \mu$ in diameter with walls $1\ \mu$ thick, and granular contents. The pigmentation forms a thick, black and uneven layer on the surface of the perithecium and fills the



TEXT-FIGURE 2.

Longitudinal section of an immature perithecium picked in mid-May. The asci are in early stages of development and the pigmentation of the cells forming the walls and the disrupted cells of the bud-scale at the base of the perithecium are shown.

intercellular spaces in this layer, but becomes progressively less intense towards the inside of the wall.

The middle layer is 19-30 μ in thickness, composed of cells of similar size and shape but with thinner walls and having less granular contents and less pigmentation. The cells of the innermost layer have thin walls, are elongated, have few stainable contents and are about 7.5 μ in depth.

The width of the perithecial wall is 47-80 μ , the average being 64.5 μ . At the apex of the maturing perithecium however, the two inner layers taper to nothing, forming a dome 30-50 μ wide at the apex. The central area consists of a single line of heavily pigmented protective cells which eventually become ruptured to form the ostiole before spore discharge takes place.

The perithecium is supported by a stalk of variable length, the surface composed of pigmented cells continuous with those of the perithecium. The two inner layers of the perithecial wall merge at the top of the stalk into cells similar to those of the middle layer. These cells form the bulk of the stalk, and gradually become less dense towards the base of the stroma, where it arises from the loose pseudoparenchyma which fills the spaces within the bud.

At the top of the stalk and directly below the sporogenous cavity is a loose mass of large, thin-walled cells up to 15 μ in diameter. Above this, forming a layer 20 μ in depth are the ascogenous hyphae which run horizontally outwards from the centre, turning upwards to form asci at the ends. The asci are embedded in a compact mass of hyaline paraphyses; these are septate, branched, and thin-walled, the cells being about 11 μ x 1 μ , often with a swelling just below the septum and a vacuole in each cell. The top of the cavity is filled with small-celled hyphae which disappear as the asci mature,

and expose the ostiole.

The ascus is roughly cylindrical, wide, and rounded at the top and tapering to a nodular swelling with a twist in the protoplasm at the base, and a narrow stalk. When mature the ascus measures 125-240 μ x 23-26 μ the walls being 4-8 μ thick and the cavity 12 μ across. The wall of the ascus is thinner at the apex enabling the ascus to rupture readily at the time of spore discharge.

The ascus normally contains eight muriform ascospores arranged in two indistinct rows and overlapping each other slightly. The spores are pale yellow in colour but become darker with age. They are spindle-shaped, tapering sharply at both ends and constricted in the middle and measure 35-58 μ x 12-27 μ , the mean of a hundred spores being 44 μ x 18 μ . The number of septa is variable but there are usually six transverse and one longitudinal septa. There are also frequently a number of oblique septa between the transverse septa.

The delimitation of ascospores within the developing ascus begins at the apex of the ascus and proceeds downwards; in some cases only six ascospores are formed, while in others small spores having one or two cells are produced from the residual protoplasm. These spores were found to be discharged with viable ascospores but not to germinate (Text - figs. 3:1,2).

When mature and about to discharge its spores the ascus begins to elongate, the walls becoming increasingly thin with the ascospores forming a compact group at the apex of the ascus. The ascus eventually ruptures at the mouth of the ostiole, the spores being explosively discharged into the air under dry conditions. Immediately following the discharge of ascospores, the ascus collapses to the floor of the perithecium. Although rare, spore discharge

TEXT-FIGURE 3.

1. Development of the ascus and the formation of ascospores.

I - III. Development of the ascus from the tip of an ascogenous hypha.

IV, V. Delimitation of ascospores from the apex of the ascus.

VI. Almost mature ascus containing some epiplasm.

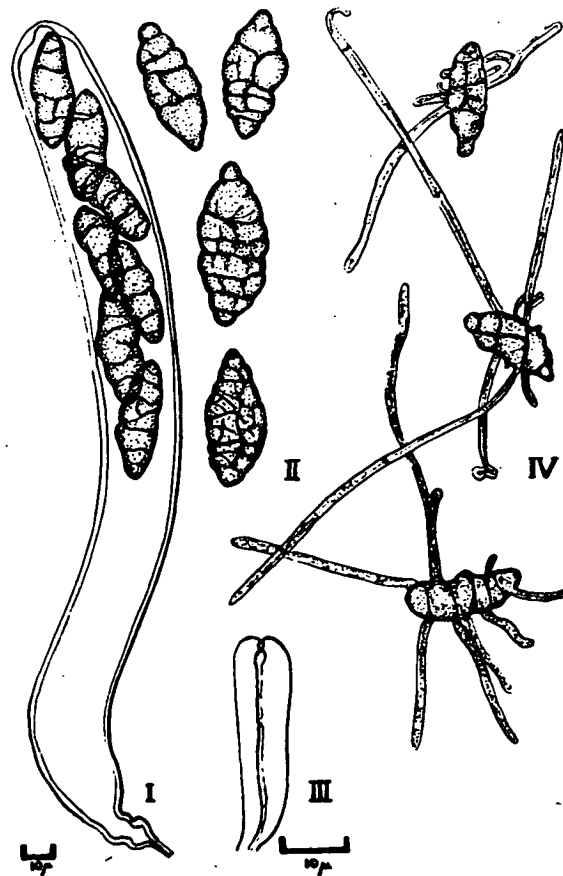
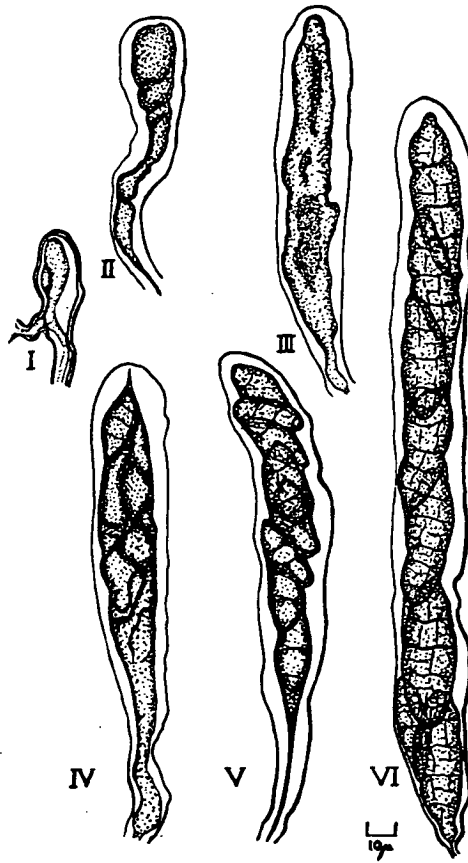
2. Discharge and germination of ascospores.

I. Movement of spores to the apex during elongation of the ascus.

II. Discharged ascospores.

III. Empty ascus (enlarged).

IV. Ascospores germinating on potato dextrose agar after 18 hours at 23°C.



1

2

is sometimes incomplete, the ascospores remaining embedded in the mucilaginous remains of the asci and paraphyses.

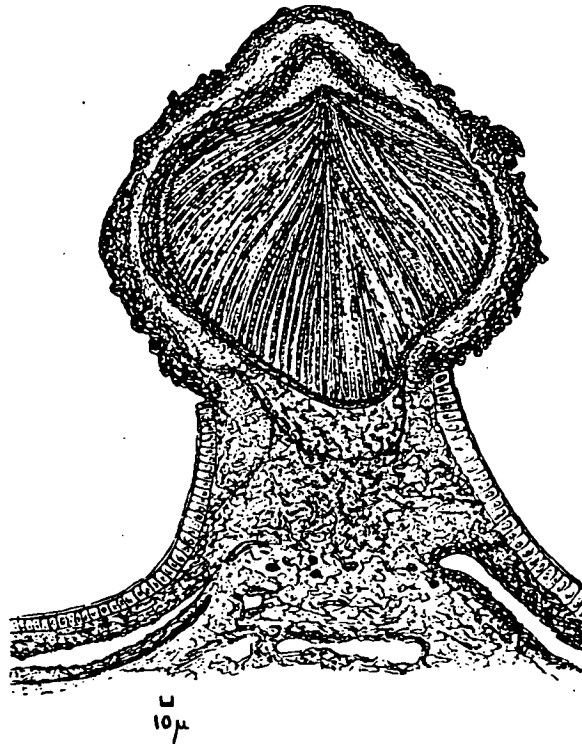
Pycnidia.

The pycnidia erupt one year after infection and on detailed examination are seen to differ from the perithecia. Externally the pycnidia appear grey-brown with a yellowish tinge in contrast to the black perithecia, but when mature they become deep brown and rough. The pycnidia also become larger than the perithecia when mature, an ostiole forming at the apex and giving the pycnidium a turbinate or pear shape from above, (Plate V , Fig. 16).

The pycnidia arise from a stroma indistinguishable from that which gives rise to perithecia, and in fact the latter appear to arise frequently on the apices of old pycnidia in the second year, or to erupt in an identical manner to the pycnidia.

A number of pycnidia were found to measure about $675\ \mu$ in length and $655\ \mu$ in width, the sporogenous cavity being $465\ \mu$ long and $435\ \mu$ wide, (Text-fig. 4.).

The wall of the pycnidium consists of two layers, the outer composed of rounded, moderately pigmented cells, $7\ \mu$ in diameter with walls $1\ \mu$ thick, and forming a layer $18\ \mu$ in depth. The inner part of the wall consists of closely interwoven, very lightly pigmented hyphae $2-3\ \mu$ wide, the total width of the wall being $47-81\ \mu$. At the top of the stalk the wall merges into a compact mass of moderately thick-walled cells $5\ \mu$ across, protected on the surface by heavily pigmented cells. In the centre of the stalk and immediately below the cavity is a mass of thin-walled cells $7\ \mu$ in diameter and $36\ \mu$ in depth. Adjoining this mass and forming a layer $11.5\ \mu$ deep on the inside of the cavity are globose, vacuolate cells $2\ \mu$ in diameter; the innermost ones, the conidiophores, are $4\ \mu$ in



TEXT-FIGURE 4.

Longitudinal section of immature pycnidium picked in early June. The pycnospores are almost mature. The pigmentation of the walls and the disrupted cells of the bud-scale at the base of the pycnidium are shown.

diameter and have a short, rounded neck on which the pycnospores are formed.

The pycnospores begin development as slightly bulbous outgrowths from the conidiophores, tapering to a point at the top and having a twist in the protoplasm near the base. As maturation advances the vacuolate protoplasm becomes separated by the formation of transverse septa, the pointed apex being left as a cap as the apical cell becomes bluntly rounded. This apical cap is later lost and at the base, the spore becomes rounded and attached to the conidiophore by a narrow neck traversed by a septum.

The mature pycnospores measure $225-337.5 \mu \times 4-8 \mu$, the average of a hundred being $283 \mu \times 6 \mu$. They are multiseptate, having about thirty transverse septa, filiform, slightly curved, bluntly rounded at the top and gradually tapering towards the rounded base. The individual spores appear hyaline, but tinged with pale brown in the mass (Text-fig. 5). The apex of the pycnidium is thickened so that the pore through which the pycnospores are discharged is broadly conical in shape, 125μ long, about 50μ in diameter at the top of the short neck and 70μ in diameter at the base. The uppermost one-fifth of the cavity and the lower half of the pore are lined with sterile cells which form periphyses. At the base of the pore, where the periphyses are about 35μ long, they almost meet: the length is only 20μ higher up the pore, whereas the pore has a diameter of 65μ , leaving an open cylinder 35μ in diameter.

Pycnospores from other sources.

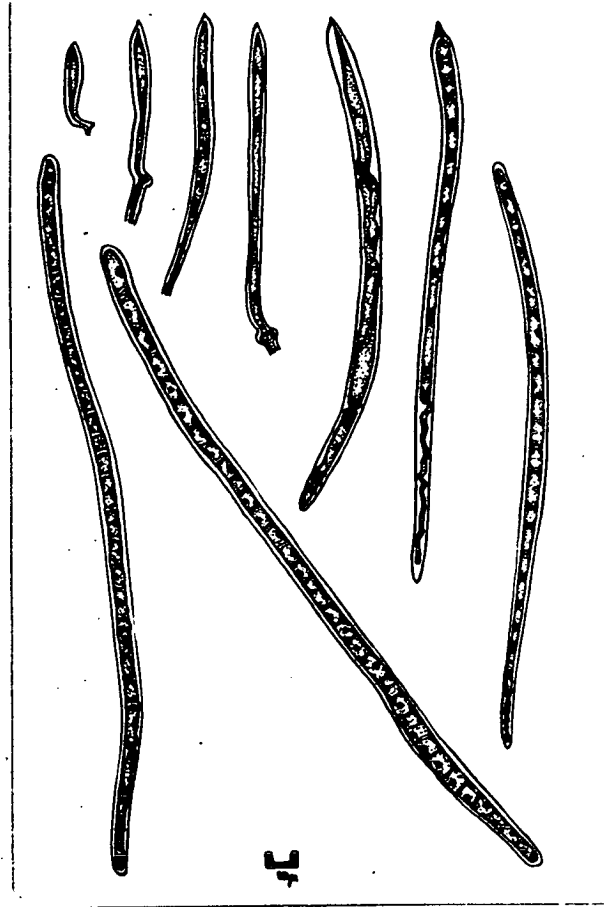
An investigation of the preserved material revealed the possibility of there being two other types of pycnospore associated with Cucurbitaria piceae. Infected buds gathered by Dr. Borthwick at Dochfour in August, and identified by him as infected with Cucurbitaria

TEXT-FIGURE, 5.

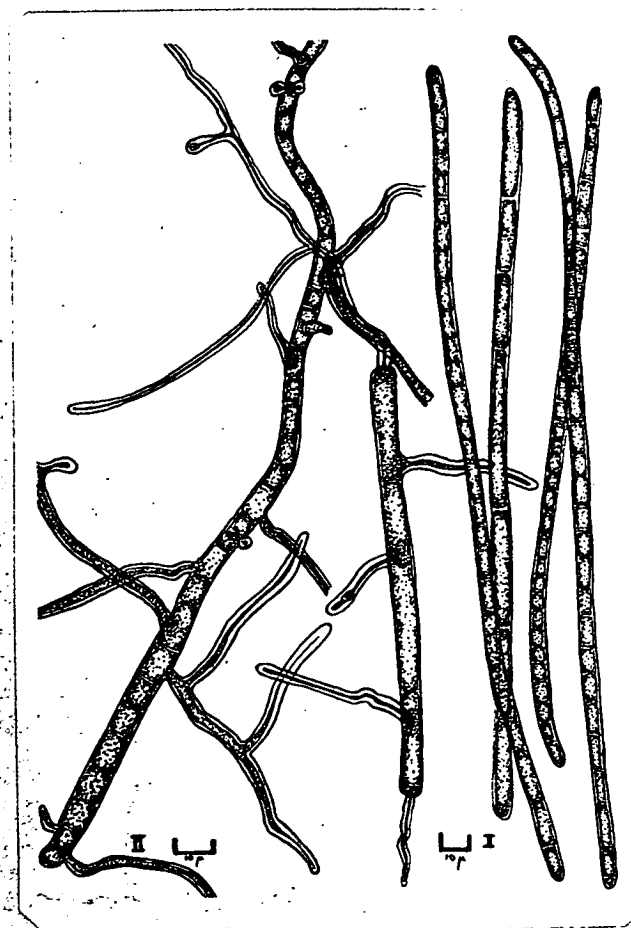
1. Development of type "C" pycnosporos showing formation of the apical cap.

2. I. Mature pycnospores after discharge.

- II. Pycnosporos germinating on potato dextrose agar after 18 hours at 18° C.



1



2

piceae, contained no asci in the perithecia. Among the limited buds however, two types of pycnospore were found in pycnidia borne on separate buds. No two types of fructification appeared to form on the same bud.

Type 'A' pycnospores were light to dark brown, regular, oval or oblong, with heavy transverse and light longitudinal septa. The spores were normally traversed by three to six septa, the longitudinal septa lying obliquely or at right angles to them. The average size of a hundred spores was $22.5 \mu \times 10 \mu$, the range being $16.5 - 31 \mu \times 7 - 12.5 \mu$ (Text-fig. 6).

Type 'B' pycnospores were straw-yellow to light brown, irregular, spherical or turbinate, with numerous septa having no definite orientation. The pointed ends had one or more transverse septa and very pale protoplasm. The range in size was found to be $19.5-35 \mu \times 13-22 \mu$, the average of a hundred spores being $27 \mu \times 17 \mu$ (Text-fig. 6).

The pycnospores of type 'A' resemble very closely those of Cucurbitaria laburni (Pers.) de Not. and the measurements agree very closely with those of that species (10). The slightly greater range in size and the presence of longitudinal septa in the Dochfour type "A" pycnospores separate these two types. Type "B" pycnospores resemble very closely those found in pycnidia in close association with perithecia of Cucurbitaria piceae in Denmark (6). Here again, however, the range in size is slightly greater; also the two types of fructification were on separate buds at Dochfour.

The type "C" pycnospores described above from Dunkeld material have been mentioned as occurring in Denmark (6), Bohemia (12) and Switzerland (15). The information from Ferdinandsen and Jørgensen (6), who give the measurements as $250 \mu \times 8-10 \mu$ and Müller (15) whose spores were $200-300 \mu$ long, agrees with Köck's description (12)



TEXT-FIGURE 6.

- A. Pycnospores type "A" from Picea pungens gathered at Dochfour in June 1924.
- B. Pycnospores type "B" from Picea pungens gathered at Dochfour in June 1924.

of the type "C" pycnospores, although in the last case they were considered to be paraphyses. The description given above agrees very closely with these earlier reports and the measurements are also in general agreement, but no description has previously been given of the development of the pycnospores or the pycnidium.

On infected buds sent by Dr. Müller from Zürich, the fructification were all found to contain pycnospores. The measurements of these spores were $137.5 - 294 \mu \times 6 - 9 \mu$, with an average of $220 \mu \times 8 \mu$. The average length is thus 63μ less, and the average width 2μ more than those from Dunkeld, while those reported from Denmark are intermediate in length and slightly wider than those from the other two sources.

In addition to shorter pycnospores, the Swiss pycnidia were slightly smaller and of a lighter construction than those from Dunkeld. The pycnidia were almost sessile, globose and $400 - 550 \mu$ in length and $550 - 650 \mu$ in width. The walls were less heavily pigmented on the outer surface, and $35 - 40 \mu$ in width.

Spore discharge and germination.

Spore discharge among the Pyrenomycetes frequently occurs after rain, and that this is so in the case of Cucubitaria piceae has been shown by observation and experiment for both perithecia and pycnidia.

Under laboratory conditions of constant warmth and high humidity, the discharge of ascospores produces a visible yellow mass at the mouth of the ostiole. This normally does not occur in the field, where surface moisture evaporates rapidly after summer rain. Under these conditions the ascospores are discharged into dry air and travel vertically for four millimetres or more. Pycnospores emerge singly from the pycnidium, slowly at first and then more rapidly as the

spore begins to taper below the middle. Another spore emerges just as the previous one is gathering speed and, in the absence of water, becomes attached to the tail of its predecessor. In this way a tendril of twelve spores may form, measuring about two millimetres in length. After moving freely for a short time the tendril becomes attached to another fructification, and a white mass of pycnospores forms at the apex of the pycnidium. In water, succeeding spores do not become attached to each other, but leaving the pore with considerable velocity, rapidly become widely dispersed.

No assessment was made of the period over which the discharge of ascospores continues. Considering the variation seen in the stages of maturity of the asci within a single perithecium, it is reasonable to assume that under prolonged and favourable conditions the discharge of ascospores would continue for several days. In addition, the perithecia mature over a period, adverse conditions lengthening this still further.

The discharge of pycnospores from a single pycnidium into a drop of water was found to continue for four hours, after which the pycnidium appeared to be empty. In these favourable conditions the entire contents of a pycnidium are shed, but the onset of a period of dry conditions leads to the accumulation of a mass of spores at the mouth of the pore. Few of these spores retain their viability after being allowed to dry for eighteen hours. Nevertheless, the production of pycnospores over a period is ensured by successive maturing of the pycnidia.

The types of spore discharge provide an interesting comparison

with those of other fungi. In this species, for example, there are no periphyses in the canal of the ostiole of the perithecium, but they do occur in the pore of the pycnidium. The function of these structures appears to be the separation of the pycnospores in order to facilitate the discharge of single spores. Unlike the asci which mature in succession, the pycnospores mature simultaneously and are ready for discharge at the same time. Among the Fungi Imperfecti the formation of a spore tendril is a common method of spore dispersal from pycnidia (11). These usually contain mucilage which raises the pressure within the pycnidium and assists discharge. In this case, the absorption of water and the shape of the spores themselves appears to promote the extrusion of the spores.

This fungus differs in two ways from other Pyrenomycetes having asci which are attached to the floor of the perithecium. Firstly, the ascus ruptures at the apex, whereas in Sordaria species the top of the ascus is shed with the spores (11). Secondly, the ostiole has no periphyses in the canal of the perithecium.

The ascospores germinate readily at 18°C on malt extract or potato dextrose agar, up to eight germ tubes being formed. Any cell of the spore may form a germ tube which soon becomes septate, branched and frequently forms loops after a short time. (Text-fig. 3:2). The pycnospores also germinate readily on malt extract or potato dextrose agar at 18°C and form up to twelve germ tubes. The terminal cells give rise to germ tubes along the axis of the spore, while any of the other cells may form germ tubes laterally (Text-fig. 5:2).

GROWTH IN CULTURE .

It has been stated above that germination of ascospores and pycnospires occurs readily at 18°C on potato dextrose agar. The colonies derived from both types of spore were compared for characteristics of colour, odour and rate of growth on several media and at several temperatures. As many colonies as possible were grown from single spores and from groups of spores, so that a wide variety of types of colony derived from both ascospores and pycnospires was available for comparison.

Media.

The media used for this work were potato dextrose agar and malt extract agar, made to the following formulae:-

Potato dextrose agar.

Potato 200 gms.

Dextrose 12.5 gms.

Agar 12.5 gms.

Distilled water 500 ml.

Malt Extract agar.

Malt extract .. 12.5 gms.

Agar 12.5 gms.

Distilled water 500 ml.

These media were sterilised in an autoclave for twenty minutes at a pressure of fifteen pounds.

Rate of growth.

Colonies derived from single spores and groups of spores of both types were maintained at 5°C, 18°C, 23°C and 32°C. The

rate of growth of both types of colony was similar on each medium at each temperature. The results are given in Table II and Text-figure 7, the figures being the means of measurements of twenty colonies derived from five or more sources.

Table II.

RATE OF GROWTH ON POTATO DEXTROSE AGAR.

Temperature °C	Culture Type	Time in Days					Average in mm/Day
		3	10	17	24	31	
5	Ascospore	1.11	5.85	12.19	18.80	25.14	0.81
	Pycnospore	0.96	5.03	10.78	17.38	24.29	0.78
18	Ascospore	7.20	21.30	34.83	46.83	55.88	1.80
	Pycnospore	6.05	20.65	34.53	48.38	59.43	1.92
23	Ascospore	1.95	8.13	15.91	24.24	31.90	1.01
	Pycnospore	2.28	9.08	17.23	25.51	33.56	1.08

RATE OF GROWTH ON MALT EXTRACT AGAR.

5	Ascospore	1.28	4.51	7.74	11.35	15.58	0.50
	Pycnospore	1.43	5.14	8.48	12.26	16.51	0.53
18	Ascospore	2.45	13.06	21.13	36.76	47.79	1.54
	Pycnospore	1.85	12.85	25.96	39.58	53.20	1.76
23	Ascospore	2.38	8.59	15.18	22.14	28.56	0.92
	Pycnospore	2.21	9.50	19.28	30.38	40.35	1.31

TEXT-FIGURE 7.

Growth rates of pycnospore and ascospore colonies
at 5°C, 18°C and 23°C.

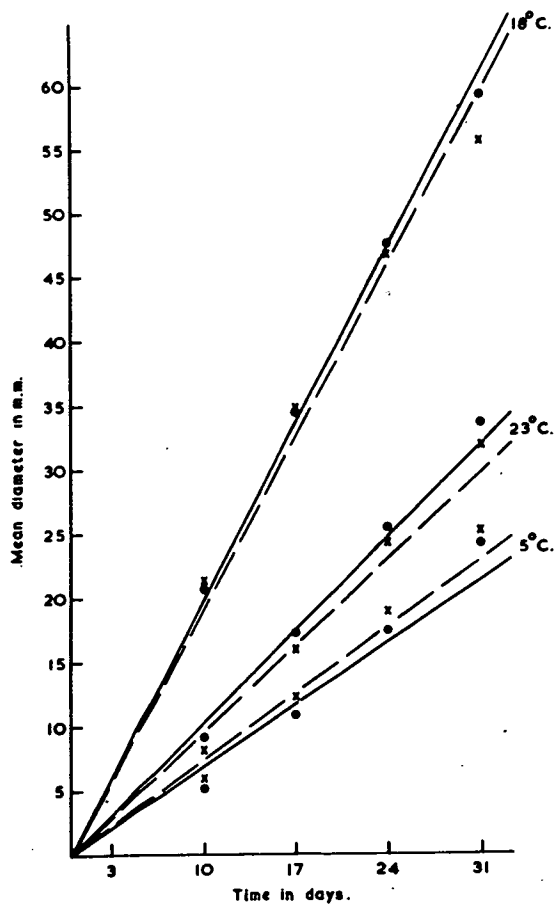
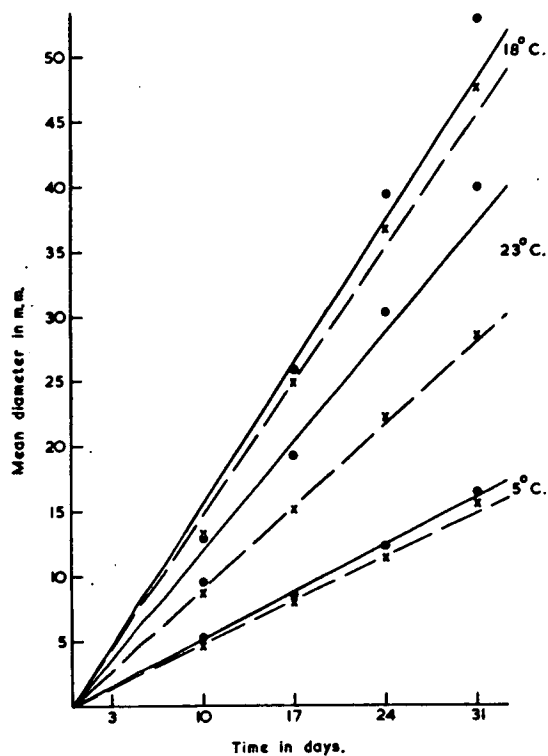
● ————— ● Pycnospore colonies.

x - - - - - x Ascospore colonies.

Above. Malt Extract agar.

Below. Potato dextrose agar.

TEXT-FIG 7



The results given show closely similar behaviour of the two types of colony on two media at different temperatures. In general the rates of growth of both types of colony on malt extract agar were slower than those on potato dextrose agar. Only at 5°C on potato dextrose agar was the rate of growth of ascospore colonies faster than that of the pycnospore colonies. On both media growth was slowest at 5°C and fastest at 18°C, fairly slow at 23°C and nil at 32°C, indicating an optimum temperature of about 15°C for both types of colony.

Colour.

Similarities in colour were found in both types of colony at all temperatures and on both media. The surface of the mycelium was white, compact and had a velvet texture. The centre was raised to form a low dome from which the colony sloped downwards, with one or more undulations to a regular and sharply defined edge.

The lower surfaces of the colonies showed wide variation on each medium and at each temperature although fundamentally they had the same general characteristics. At 18°C on potato dextrose agar there were three main groups, each embracing both types of colony. The basic colour was neutral gray, ranging from mouse gray through olive gray to gull gray. Outside this was a ring of sepia brown ranging from Hay's maroon through olivaceous black to iron gray, and a second ring of olive brown ranging from cinnamon drab through light brownish olive to dark greenish olive. The edges were Tilleul buff, deep olive buff or pale olive buff (17).

Variations in colour were due mainly to differences in width of the rings which corresponded to undulations on the upper surfaces of the colonies. In some colonies the entire lower surface was

of one deep colour with a narrow buff margin, while others showed no deep colour and were predominantly buff. The former type had deep, compact mycelium with dark hyphae penetrating to the lower surface of the agar which was darker towards the centre. The pale colonies had sparse, pale mycelium growing mainly on the surface of the agar and caused no darkening of the medium. Similar colours were observed in colonies grown on malt extract agar at 18°C, but here the basic colour was browner. Colonies grown on malt extract agar were basically olive brown in colour at all temperatures whereas those on potato dextrose agar were greenish olive. At 5°C the colonies on both media were more intensely coloured, and the upper surface of the mycelium was pale grey-green; these colours were less intense at 23°C. Staling was very pronounced after thirty-one days at the higher temperatures, the centre becoming smooth and waxy. Some colonies became uneven towards the edge and deep hollows separated compact tufts of mycelium joined by strands of hyphae. The hollows gradually became bigger and frequently joined the central staled area as the colonies became older.

The variations in colour described were greater between colonies derived from one type of spore than between colonies derived from both types. and it was found to be impossible to separate colonies on this character. Odours produced by both types of colony were also too similar for distinction to be made by this means. On potato dextrose agar the colonies had an earthy, musty odour, especially at 5°C, while those on malt extract agar had an odour of fermented malt (Plates IX-XIV).

Special media.

A number of colonies were grown on Spruce agar and a Spruce sawdust medium in an attempt to produce fructifications in culture. The Spruce agar was prepared by grinding buds, leaves and young stems in a pestle and mortar with distilled water, and adding to agar and water as follows:-

Spruce	6 gms.
Agar	4 gms.
Distilled water..	250 cc.

A sawdust medium was composed of moist Spruce sawdust to which five percent of "accelerator" had been added (1a). This was put into Petri dishes and also packed in test tubes inserted in flasks as described by Badcock (1b).

A number of inoculations with mycelium from ascospore and pycnospore colonies were made. One tube of Spruce agar inoculated with mycelium from a single ascospore on April 24th and kept at room temperature until dry formed small black bodies on the surface by Oct. 12th. Microscopic examination showed these to consist of compact masses of hyphae, but no structure typical of pycnidium or perithecium was observed. A flask inoculated with ascospore mycelium on January 29th 1952 showed formation of mycelium at the base of the tube by the end of 1953 but no fructifications developed. The tube was removed and exposed to daylight while being kept damp. By October 1954 luxuriant growth of mycelium had developed on the surface of the cotton wool, but no fructifications had been formed.

All other attempts to produce fructifications on sawdust, spruce extracts and special media, intermittent temperatures, freezing (7) and changes of nutrition proved unsuccessful.

MISCELLANEOUS LABORATORY EXPERIMENTS.

1. A number of young shoots of Picea pungens were placed in test tubes, the base of the shoots resting on agar. After sterilisation the agar was inoculated with mycelium from ascospores and tissues removed from infected buds. After seventeen months the hyphae had invaded all tissues except the wood, causing them to become soft and brown. No fructifications developed but black lines were formed at the tips of needles, where they touched the sides of the tube.
2. Sections of infected and uninfected Spruce buds were tested for the presence of cellulose, by placing sections of iodine solution under a cover slip and allowing 75% sulphuric acid to diffuse inwards (11a). The blue colour thus formed showed that in an infected bud cellulose was entirely removed from the needles and bud-scales, only the outermost bud-scale remaining intact. The remainder of the bud-scales and the needles were reduced to disorganised masses of lignified tissues and resin droplets.
113. A number of tubes were partly filled with agar containing 0.007% gentian violet (16a) and inoculated with mycelium from ascospores and pycnospores. Two tubes showed no loss of colour after three months, and six showed some, of which two were ascospore and four pycnospore colonies. This loss in colour is characteristic

of wood-rotting fungi causing white rot, and indicates some ability on the part of the fungus to live on all the tissues of the bud, since it has also been shown to remove cellulose from bud tissues.

4. Both ascospores and pycnosporos germinated readily on malt extract agar, potato dextrose agar and 2.5% agar. In distilled water, however, germination was confined to the formation of short germ tubes.

Freshly-cut Spruce stems were soaked in distilled water for four days and the water then used for germination tests at 10%, 50%, and 100%.

The length of the germ tubes formed after forty-eight hours was greater in 10% extract than in distilled water. In 50% extract the amount of growth was much greater, and the formation of hyphae was very advanced in 100% extract.

SOME ASPECTS OF THE HOST/PARASITE RELATIONSHIP.

In addition to the detailed examination of the fructifications, a study of infected material was made with particular reference to the locus and method of infection, the depth of penetration of the fungus into the tissues of the host and its means of nutrition.

In addition, an assessment was made of the proportion of infected buds, the damage done and the effect on the growth of the host.

Locus of infection.

The buds of the Spruce, especially those of the Blue Spruce which have recurved bud-scales, provide an ideal site for the lodgement and germination of spores. Field experiments have shown that infection may follow the application of spores to the apex of a newly formed bud, and that no infection occurs if young buds are covered during the period of spore discharge.

These results, together with the fact that microscopic examination of numerous shoots bearing infected buds has revealed no hyphae within the tissues of the stem, lead one to the conclusion that infection normally occurs at the apex of a young bud.

Method of infection.

Examination of buds picked in September showed hyphae extending between the bud-scales from the apex to the base. The hyphae were few in number and had few branches between the bud-scales but formed a sheath over the embryo shoot. Hyphae penetrating further into the base of the bud formed a loose mycelium in the resin canals.

It seems therefore that following the germination of a spore at the apex of a young bud, the germ tubes grow between the bud-scales towards the base. The hyphae later enjoy the protection afforded by the resinous bud-scales during the winter months, although at the time of infection the formation of the layer of resin has barely begun. The fungus first attacks the shoot at the apex of the young shoot

Depth of penetration.

Microscopic examination of undeveloped shoots contained within infected buds revealed the occasional presence of hyphae in the medulla, the affected cells being isolated by the formation of a cork layer at the limit of the living cells (Plate IV, Fig. 11). A complete absence of hyphae within the medulla was shown by young shoots having a complete cork layer, indicating the efficacy of these cork cells as a barrier to the spread of the fungus.

A natural barrier of this type is to be found in the absciss layer at the base of the deciduous bud-scales. This layer, which is partly composed of cork cells protects the cortex when the bud-scales are forced off in the spring.

It has already been observed that no hyphae appear to invade the tissues of the stem, and the presence of an obstacle to the spread of the fungus lends support to the conclusion that it is contained within the limits of the bud.

Nutrition.

The appearance in May of shoots invested with grey mycelium has been described. The masses of cells contained within the young buds in the previous autumn, from which these shoots had developed, were covered by a loose mycelium in September. At this time a few intracellular hyphae were also present in the cortex, but in view of the fact that the majority of infected buds tended to give rise to shoots, the presence of the fungus can only have had a very limited effect on the normal processes of development. Vegetative development appears to be very slow, the fungus first drawing its nourishment from the living cells of the young shoot

and later from the dead tissues and the inner bud-scales.

Although a small number of buds were found which remained alive for two years, the majority were killed by the end of the year following infection. Under these conditions the fungus would change from a parasitic to a saprophytic mode of nutrition about this time.

The genus Cucurbitaria is commonly saprophytic during the perithecial stage and it has already been pointed out that only pycnidia are produced during the first year, and that perithecia appear only in the second and later years.

If, therefore, the type of fructification to be found on the surface of the bud is taken in this case as a sign of the nutriment available within the bud, the fungus can be seen to live parasitically for almost eighteen months. The presence of pycnidia alone in the first year is followed by the appearance of perithecia in the second, reflecting a moribund condition of the tissues and the change to a saprophytic existence. The death of the bud is marked by the formation of perithecia alone after two years.

Extent of damage to buds.

One of the characteristic symptoms of infection by Cucurbitaria piceae is the formation of twisted buds, and in order to establish whether any relationship existed between the degree of contortion and the severity of the infection, a count was made of the buds on material gathered at Dunkeld on September 23rd 1950.

The results are given in Table I. As this figure also indicates the proportion of buds which remained sufficiently

Table I.

Number of uninfected, infected and distorted buds in September 1950.

<u>Uninfected buds</u> (<u>no fructifications</u>)		<u>Infected buds</u> (<u>bearing fructifications</u>)	
Total number	408	Total number	937
Percentage of total buds	30.3	Percentage of total buds	69.7
Newly formed buds.....	215	Untwisted buds (few fructifications)	774
Percentage of total buds	16.0	Percentage of total buds	57.5
Percentage of uninfected buds	52.7	Percentage of infected buds	82.6
Dormant buds	193	Twisted buds (many fructifications)	163
Percentage of total buds	14.4	Percentage of total buds	12.1
Percentage of uninfected buds	47.3	Percentage of infected buds	17.4
Number of branches examined ...		36	
Number of buds examined ...		1345	

It can be seen from Table I that in this instance the twisting of buds was not a general character of the disease, only 17.4% of those infected showing any distortion. As this figure also indicates the proportion of buds which remained sufficiently

active for some growth to take place in the spring, almost 83% had been killed by that time.

In order to make an assessment of the damage caused by this fungus on the trees at Dunkeld, a study was made of the buds of Picea, the sequence of events that accompanies the termination of dormancy and the formation of new shoots during the annual period of growth. The resting buds are protected by true bud-scales, most of which arise from a ring of primary cortex, within which the dormant bud is situated. The remainder of the bud-scales, which are larger and persistent, arise as a continuation of the cork layer of the stem. With the start of annual growth within the tissues of the bud, the shoot elongates and exerts pressure on the bud-scales causing a rupture at the absciss layer round the base of the cortical ring. As growth continues, the ring of tissue, together with the bud-scales, is carried up on the apex of the new shoot, to be ultimately discarded. When a bud has become infected the hyphae bind the cap of deciduous bud-scales to the outer persistent scales and prevent its removal when seasonal growth begins. The variation found among infected buds is therefore due to the amount of growth that the young shoot is able to achieve in the year following infection. A small proportion of buds, as pointed out above, retain some activity until the spring, and here it is not so much the presence of the fungus within the tissues, as the restriction it places on the normal growth processes, which controls the degree of abnormality finally shown by the bud.

This was borne out by a microscopical study of the changes

which take place within the bud after infection.

Infected buds were picked in early autumn and those in which limited growth had taken place were fatter than normal and flat-topped. A varying number of fructifications was present on the bud-scales, their development proceeding downwards from the apex of the bud. Entirely contained within the bud was a rudimentary shoot bearing minute needles and a terminal bud surrounded by a shallow ring of cortex with a few bud-scales. The surface of the shoot was closely covered with dense pseudoparenchyma which extended between the bud-scales and filled all available space within the bud. In the absence of normal elongation and expansion the cells of the cork layer had become folded and the cortex abnormally thick. In the event of the fungus having penetrated into the medulla, the affected area was isolated by the formation of a layer of cork cells. Tissues outside this barrier were disorganised and broken down. (Plate IV, Fig. 11).

Severely distorted buds in which the bud-scales were split by twisting were often found to have the underlying tissues exposed. Sections of these buds showed that in many cases no wood was present, the cells of the medulla being exposed.

In considering the damage done to those buds not immediately killed by this fungus, that done to rudimentary shoots, where these remain enclosed within the bud-scales, has also been described. In addition to these, a number of infected buds give rise sooner or later to shoots which, although deformed, bear green needles and become part of the branch system of the tree.

Damage to shoots.

Shoots arising from infected buds vary in length from a few millimetres to several centimetres by the end of the summer. The

appearance of shoots which only partly emerge from the bud has been described. These shoots usually bear about a dozen small needles on one side.

In cases where the shoots burst out of the buds, they frequently do so laterally. The stems of these shoots are short and thick, due to the presence of extra vessels in a radial direction, and often twisted (Plate I, Fig. 3).

The infection of a lateral or subterminal bud usually prevents the subsequent growth of a shoot, although a shoot may form from an infected terminal bud. Where vigorous growth occurs from uninfected subterminal or lateral buds, the terminal bud rarely gives rise to a shoot (Plate I, Fig. 2 and Plate II, Fig. 4), but where every bud on a branch has become infected, a moderately normal ^{terminal} shoot may develop (Plate I, Fig. 1). Shoots may form, however, which bear few needles, and can play no further part in the formation of new branches (Plate I, Fig. 3).

It can be seen that with such irregularities in the formation of shoots, there must be some reduction in the number of needles borne on the tree, and a consequent loss of vigour. With each year the condition of the tree would deteriorate rapidly but for the formation of additional buds able to form new growth.

Formation of axillary buds.

Much of the new growth was found to result from buds formed in the axils of the persistent bud-scales. These remain for some years, and as many as five axillary buds were present at the base of one shoot. It appears that these buds form two or three years after the loss of needles is probably accelerated in this way by the fact that the cessation of growth further along the branch, and being further that the stem no longer performs any function in the passage of materials to and from the growing point of the branch, so that the whole branch soon dies.

than the new buds of the current year.

The shoots formed from axillary buds, together with those which arise from lightly infected buds compensate to some extent for the absence of the normal annual formation of new shoots.

Effect on growth of the host.

The interruption to the normal elongation of branches which follows infection by this disease, is accentuated by the fact that it is the terminal and subterminal buds which form the focal point of the attack. Although some growth occurs on infected branches, a redistribution of growth forces otherwise dormant buds into activity. In consequence, individual branches show growth patterns which mimic those normally associated with other trees.

Repeated infection of the terminal bud followed by growth of a single subterminal or lateral bud results in a branch system imitating a sympodium (Plate III, Fig. 8). If the infection of the bud has been followed by equal growth from two subterminal buds, the branches show false dichotomy (Plate III, Fig. 9). Again, where new growth is entirely due to the activity of axillary buds, whorled branches typical of the main axis of Picea are produced (Plate III, Fig. 7).

Occasionally gradual defoliation appears to follow the complete cessation of growth on a branch. There is no evidence that this is due to the presence of this fungus in the stem, and a study of the branches affected in this way showed that no growth had taken place on them for at least two years. The needles only live for a few years and defoliation normally follows after a given interval. The loss of needles is probably accelerated in this case by the fact that the stem no longer performs any function in the passage of materials to and from the growing point of the branch, so that the whole branch soon dies.

As a result of infection by this disease then, the spread of the branches is restricted and the tree assumes a more compact habit than is normal. It must be borne in mind however, that the infection at Dunkeld has been comparatively light so far, and confined to the lower branches. Here, considerable new growth is added each year in spite of the presence of this fungus. In epidemics of the magnitude of those described by Jørgensen in Denmark, virtually all growth is suppressed and death may eventually follow.

FIELD AND LABORATORY OBSERVATIONS.

Visits were made to the infected trees at Dunkeld on the following dates: September 23rd, 1950; April 16th, May 10th and October 15th, 1951; April 20th, May 18th, June 11th and August 27th, 1952 and March 31st, 1954. Observations made in the field and on fresh material collected for laboratory examination are summarised below.

In April, infected buds bearing fructifications discharged viable ascospores after being kept warm and damp in the laboratory for ten to twenty days. Pycnospores were discharged after nineteen

In April and May the infected buds could be distinguished by their abnormal development or failure to burst. Buds were found to be curved to varying degrees, the result of a struggle by the developing shoots to emerge from buds bound together by mycelium in such a way as to prevent the normal removal of the dehiscent bud-scales. In extreme cases the bud-scales were split down one side exposing the developing shoot, the outer tissues of the stem also frequently being ruptured to expose the wood. The needles on these shoots were brown, withered and closely bound together by grey mycelium.

Some shoots had emerged, however, by bursting laterally through the bud-scales; these were squat and of greater girth than is normal. Abnormal shoots also developed from buds infected two years previously, and bearing fructifications. In these cases the shoots frequently emerged terminally or laterally from the infected buds, or from new buds formed in the axils of the persistent bud-scales. It is thus evident that the presence of mycelium within the bud does not necessarily imply the permanent cessation of meristematic activity.

Abnormal behaviour in the spring was followed during the summer by the appearance of fructifications towards the apices of the infected buds. The first of these matured and discharged pycnosporos by August, while others continued to form. In the following spring the bud-scales were thickly covered with maturing pycnidia and perithecia which became distinguishable only at the time of spore discharge.

In April, infected buds bearing fructifications discharged viable ascospores after being kept warm and damp in the laboratory for ten to twenty days. Pycnosporos were discharged after nineteen

to twenty-six days; in May this period was reduced to five days. In June 1952, buds infected in 1950 showed discharged pycnospore masses at the apices of pycnidia and discharged spores immediately after wetting.

During the second summer after infection, only ascospores were discharged.

INFECTION EXPERIMENTS.

Infection experiments were carried out at Dunkeld on June 11th, and at the Department of Botany on June 12th 1952.

At Dunkeld all buds bearing fructifications were removed from seven heavily infected branches. The ends of the branches were then immersed and agitated in distilled water and enclosed in cellophane bags perforated on the lower side. The covers were removed on August 27th 1952 when the period of active growth was over, and the newly formed buds were protected by stout bud-scales. Each of the buds thus covered during the period June-August 1952 gave rise to a healthy shoot during the summer of 1953, and no fructifications have since appeared on any of them. In addition, two healthy axillary shoots arose during 1953 from the base of an excised terminal bud.

The second experiment, carried out at Edinburgh, consisted of placing spores on the apices of selected buds and enclosing the shoots in celluloid tubes closed at each end with a cotton wool plug. After 18 days the plugs were removed, the tubes in some cases being left over the shoots for a further period before removal.

Infection with ascospores was attempted in two ways. In the first, germinating ascospores were removed from malt extract agar and placed in a small quantity of distilled water and agitated gently. Droplets were then placed at the apices of a number of buds on three

branches, and the shoots covered; owing to the resinous condition of the bud-scales, the water droplets were found to run off very readily. Secondly, therefore, five selected shoots were sprayed with distilled water by means of an atomiser until thoroughly wet, and germinating ascospores on small pieces of malt extract agar were then placed on the tips of sixteen buds and the shoots covered.

Pycnospores were placed on thirty buds in the same manner as that just described, and also as a spore suspension using spores from Dunkeld and from Zürich. In addition, an infected bud bearing pycnidia from Switzerland was attached to a shoot, sprayed with distilled water and covered.

One of the twenty-six buds treated with a suspension of pycnospores from Dunkeld failed to burst in the spring in 1953, and when subjected to detailed examination in October, was found to show symptoms typical of infection with this disease. The fructifications were all of the pycnidial type.

While these results offer no proof of any connection between the two spore types used they are in general agreement with the observations previously described.

The absence of infection on shoots covered during the period June-August suggests that there is no spread of hyphae beyond infected buds, and therefore supports the conclusion that infection by means of pycnospores may occur in June at the apex of a bud.

THE EFFECT OF CLIMATE ON THE LIFE-HISTORY.

It has been suggested by Tubeuf (20) that death or injury of the buds by frost might predispose them to infection by Cucurbitaria piceae. Picea pungens is susceptible to frost damage, the buds becoming distorted in a manner similar to that caused by infection with this fungus (2), whereas Picea excelsa is not. No evidence of frost damage was found on Picea pungens at Dunkeld although the winter temperatures are as low as those in Denmark where Picea excelsa has been severely damaged by Cucurbitaria piceae.

It has been shown that infection of the newly formed buds may occur in June, and it therefore seems that infection by this fungus is in no way influenced by frost damage. The climatic factor having the greatest influence is undoubtedly the rainfall during the critical period of spore discharge and infection.

A very heavy infection occurred at Dunkeld in 1948, resulting in the death of most of the buds (Plates I, III). No further growth took place on a number of branches, and gradually defoliation and death followed (Plate III, Fig. 9). Some recovery was achieved in 1949 and 1950 when light infections took place and a number of subterminal and lateral or axillary buds gave rise to new growth.

It is significant that the summer and annual rainfall recorded at Dunkeld during the period 1946-1952 was highest in 1948 when the heaviest infection occurred, and lowest in 1949 when the infection was very light. During the months May-August, a heavy rainfall in June has a greater influence on the severity of infection than in other months, and this is especially so when May is wet. The

weather in July and August appears to have little effect on the fungus, although a high rainfall during early July must be advantageous in the event of delayed spore discharge. In 1948 for example the rainfall was 50% above the normal in May-July and over twice the normal in August, so that the dispersal of spores and the chances of infection were increased enormously. In 1949 on the other hand, the rainfall in both May and June was a little below normal and in July was 74% of normal.

It is, however, not merely the amount of rain which has an effect on infection, but frequency with which a measurable amount of rain falls. In June, 1948, 0.01 inches was recorded on 20 days (121.7% of average) and 0.04 inches on 16 days (145% of average). In June 1949, 0.01 inches was recorded on 11 days (67% of average) and 0.04 inches on 7 days (63% of average). In May 1948 and 1949 the records were 148% and 120% of average respectively. Although some latitude inevitably exists in any correlation between rainfall and the severity of infection, a definite trend can be seen.

The preference of this fungus for moist conditions is shown by its geographical distribution (Text-fig. 1). In Scotland and Germany the localities in which the disease has been reported are all within the shelter afforded by a river valley and at an altitude of less than 600 feet above sea level. Those in Denmark are below 600 feet and close to rivers, fiords or the sea, while those in Bohemia, Switzerland and the Tyrol are situated within river valleys or catchment areas at altitudes of 1,500 - 3,000 feet.

It appears, therefore, that Cucurbitaria piceae is more or less restricted to areas of high atmospheric humidity and as has already been mentioned, the disease was much more widespread on the sheltered branches of the trees at Dunkeld, than on those exposed to sun and wind.

The advantages of high humidity must outweigh any disadvantages due to the increased likelihood of frosts consequent upon the fungus' choice of locality. Any influence that low temperatures may have on the fungus must be small, since it has been shown that it grows well in culture at 5°C, and previous damage to the host is not a necessary precursor to infection.

D I S C U S S I O N

The publication in 1909 of a paper reporting the discovery in Scotland of a new species of Cucurbitaria, was followed by a lapse of eight years before the disease was again reported. At intervals during the next thirty years, other reports came from Scotland and Central Europe, Germany and Denmark. The study of the order in which these reports were published, gives no indication of the place of origin, or of the subsequent spread of the disease. Its appearance on the upper branches of mature trees of Picea excelsa in Scotland (21) and Denmark (6), may have passed unnoticed for a number of years, in spite of the severity of the attack in the latter country. This could possibly be due to the fact that in forest plantations an attack in the upper branches would come to light only when mature trees were felled for timber. In the case of attacks on Picea pungens, however, the possibility of the fungus being overlooked is more remote since, not only has this species of Spruce a purely decorative value, but in every locality it has occurred on the lower branches of young trees, or on seedlings.

It seems unlikely however, that the disease originated on a single tree in a private estate in Perthshire, and that within forty years, it has spread to scattered localities in Central Europe and Denmark. The sporadic nature of the occurrence of this disease tends to obscure any evidence of radiation from a central point of origin. From the reports of its discovery, it seems likely that it was to be found in Central Europe at the time it was discovered in Scotland. In view of the wide distribution in Central Europe in 1918 (20), it seems possible that the disease originated in Europe, and was introduced

into Scotland on imported seedlings. The satisfactory solution to this problem could come only from a study of complete records of the history and origin of the infected trees, in every locality in which the fungus has been observed.

The derivation of Cucurbitaria piceae is also of interest. If it was first differentiated as a species as recently as 1900, it seems reasonable to assume that it arose as a variation of a species of Cucurbitaria known to have existed before this time. In this connection, Tubeuf suggested that Cucurbitaria pithyophila (Kunze et Schw.) might have been the species from which this disease was derived (20). Prior to Borthwick's discovery of the new species, Cucurbitaria pithyophila was the only species of the genus to be found on coniferous trees. Abies pectinata, which was frequently attacked by this fungus has only once been reported to be the host of Cucurbitaria piceae (6). Although the ascospores in both these species are muriform, those of Cucurbitaria pithyophila measure $16 - 23 \mu \times 6 - 8 \mu$ (16), whereas those of Cucurbitaria piceae from all sources measure $43 - 50 \mu \times 14 - 18 \mu$. According to Welch (22), the nearest approach to ascospores of this size in species of Cucurbitaria, is found in those of Cucurbitaria ulmicola (Fuckel) found in America. The ascospores in this species measure $46 - 48 \mu \times 15 \mu$, whereas in Europe they measure $36 \mu \times 14 \mu$. In view of this difference between the above mentioned species in respect of a character as constant as that of the size of the ascospores, it seems more probable that Cucurbitaria piceae represents the perfect stage of a fungus hitherto placed in the Fungi Imperfecti, than a new species derived from a pre-existing one.

It has been suggested in this connection that Camarosporium

laburni is the imperfect stage of Cucurbitaria laburni (22,18). Jørgensen subscribed to this theory by describing the discovery of pycnospores of a Camarosporium type in pycnidia associated with the perithecia of Cucurbitaria piceae in Denmark. Similar spores were found on preserved material gathered at Dochfour, and assigned by Borthwick to Cucurbitaria piceae. Examination of this material during the present study also revealed the presence of a second type of pycnospore resembling those of Cucurbitaria laburni.

In his investigation of this disease, Jørgensen also found another type of pycnospore in pycnidia externally identical to, and in intimate association with the perithecia. Pycnospores of this type were also found by Müller in Switzerland (15), Kück in Bohemia (12) and by the author at Dunkeld. These spores cannot readily be assigned to any known species of fungus, and proof from cultural or infection experiments that these are the imperfect stage of Cucurbitaria piceae, is at present still lacking. It is suggestive, however, that spores of this type should have occurred in close association with Cucurbitaria piceae in four countries, while other types of pycnospore have occurred rarely and in one case on different buds to the perithecia. Should these type "C" pycnospores prove to be the imperfect stage of this fungus, some light may be shed on the imperfect stage of the genus as a whole. The presence of pycnospores in some species of Cucurbitaria has been reported (8), but it appears far from certain that pycnospores typify this stage in all species of the genus.

The fact that the species of the host varies from one locality to another, that the disease often occurs on only one species in areas where alternative hosts are available, and that the form taken by the attack differs with the host, points to the possibility that

there may be more than one specific strain of Cucurbitaria piceae. In addition, the effect of this fungus varies considerably, not only from one area to another, but between adjacent trees. In an isolated group of trees, such as those at Dunkeld, it can reasonably be assumed that they have all been infected by the same strain of the fungus. Nevertheless, two of the trees show the effects of attack by this disease much more clearly than the remainder. It is probable therefore, that a variable degree of physiological resistance to disease exists among trees of the same species, and also between one species and another. This resistance to the fungus would result in the variation shown in the severity of the attack, but would not account for the different forms shown by the fungus in its attacks on different species of host.

There has been some difference of opinion as to the time at which the spores of Cucurbitaria piceae are shed. Borthwick found that although there were no asci in May, material kept in water until August produced abundant ascospores. Ferdinandsen and Jørgensen however, were of the opinion that infection occurred between November and April. It has been established in the course of this study that discharge of spores follows periods of rain, a general character amongst the Pyrenomycetes, and that in Scotland and Switzerland it takes place in June or early July. The ascospores mature first and discharge begins about ten days before discharge of the pycnospores begins. The discharge of spores occurs at a time when the presence of newly formed buds unprotected by resinous bud-scales provides a ready means of entry. This suggests that infection occurs at about the end of June in all localities, since the time at which the new buds are formed by the host does not vary greatly.

As a result of the almost simultaneous production of two types of spore, each capable of dispersal under different conditions, dissemination can follow both brief and prolonged periods of rain. In Scotland at least, the severity of infection was found to be influenced to a considerable extent by the June rainfall. The value of moisture to the fungus is also indicated by the frequency of its occurrence within the shelter of river valleys where the highest possible humidity is likely to occur. In favouring low-lying situations the fungus is especially liable to be subjected to low winter temperatures, but its ability to grow at such temperatures has been shown. If temperature plays any part in the life-history of the fungus, it is in initiating the sexual phase once the change from a parasitic to a saprophytic existence has taken place. As this phase occurs only after the second winter, the change must be due almost entirely to a change of nutrition, possibly assisted by the killing of the damaged host tissues by frost.

Ferdinandson and Jørgensen (6) suggested that the fungus grew saprophytically on the bud-scales becoming parasitic later. It has been found that in September, hyphae are present within the bud as a loose mass covering the growing point of the embryo shoot, the bud-scales showing no signs of destruction. In the following May the needles are brown, withered and encased in a felt of mycelium. Other Sphaeriales show a change from parasitism to saprophytism prior to sporulation (19) and the genus Cucurbitaria was held by Gray to be saprophytic on wood, at least in the perithecial stage (9). That this is probably true for this species is indicated by the appearance of perithecia in the second year, reflecting the nutritional changes within the bud. The breakdown of the inner bud-scales and the

tissues of the undeveloped shoots would provide the food material necessary for the sexual phase.

This fungus is, therefore, to be regarded as a facultative saprophyte producing pycnidia during the parasitic phase and perithecia during the saprophytic phase. The assumption that the pycnidia represent the imperfect stage of this fungus seems to be justified in spite of the lack of proof. Infection experiments gave inconclusive results, but comparison of cultures derived from ascospores and pycnosporos showed closely similar characteristics in colour, rate of growth and odour.

Pycnidia of the type described are found among the Fungi Imperfecti in the Order Sphaeropsidales, (Sphaerioidaceae-Scolecosporae) which contains a number of plant parasites. The discovery of the perfect stage among these fungi frequently leads to their transference to the Pyrenomycetes, so that pycnidia of this type could reasonably be expected as the imperfect stage of Cucurbitaria piceae.

Control of Cucurbitaria piceae could best be achieved by the application of a fungicide early in June. Since both types of spore are produced in a comparatively short space of time, one or two applications should be sufficient to provide protection during the infection period. Unpublished work by Dr. E. Müller in Switzerland has shown that a fungicide applied late in June can give good control of this disease.

SUMMARY.

1. The origin and derivation of this disease were obscure and the existence of more than one strain was probable. The geographical distribution suggested that the fungus was present in Europe at the time that it was first reported in Scotland, and might have been spread on exported seedlings.

The disease was confined mainly to the shelter of river valleys at moderate altitudes, the entire area of distribution having a moderate climate with fairly high rainfall. Frost had no apparent effect on the fungus.

2. Examination of fructifications at Dunkeld, Perthshire, showed the presence of pycnidia of a type found in three other countries. Infection experiments with pycnospores produced symptoms typical of infection by ascospores of C. piceae. Pycnidia were found in intimate association with perithecia and discharged spores about two weeks later. Ascospores were forcibly discharged under drying conditions. Pycnospores were discharged under wet conditions forming tendrils which rapidly dispersed.
3. Spore production in the field occurred during June or July and exceptionally wet summer weather resulted in unusually heavy infection. Infection following inoculation in June took place at the apex of a newly formed bud, whence the hyphae penetrated into the living tissues within the bud. Penetration was limited to the bud by the absciss layer.
4. The restriction on the normal growth processes and the inability of the bud to burst in the spring, resulted in the twisted buds characteristic of this disease.
5. The majority of infected buds were killed by the spring

following infection, pycnidia forming late in the first summer. The formation of perithecia occurred only in the second and later years, the change reflecting the onset of the saprophytic phase.

6. Comparisons were made of colonies derived from ascospores and pycnospores grown at several temperatures on two media. Rates of growth, colour and odour were found to be so similar that separation by these criteria was impossible.
7. The development of pycnidia and pycnospores was studied in detail. These spores were distinct from those of any other known species of the Fungi Imperfecti.
8. The application of a fungicide in early June was suggested as a means of control of this fungus.

REFERENCES.

- 1 (a). BADCOCK, E.C.(1942). New methods for the cultivation of wood-rotting fungi. Trans. Brit. mycol. Soc., 25, 200.
- (b) ————— (1943). Methods for obtaining fructifications of wood-rotting fungi in culture. Trans. Brit. mycol. Soc., 26, 127.
2. BAXTER, D.V. (1943). Pathology in Forest Practice. J. Wiley & Sons Inc., New York.
3. BORTHWICK, A.W. (1909). A New Disease of Picea. Notes from the Royal Botanic Garden, 4, 259.
4. DALLIMORE, W. and JACKSON, A.B. (1923). A handbook of Coniferae including Ginkgoaceae. Edward Arnold & Co., London.
5. DENNIS, R.W.G. and FOISTER, C.E. (1942). List of diseases of economic plants recorded in Scotland. Trans. Brit. mycol. Soc., 25, 266.
6. FERDINANDSEN, C. and JØRGENSEN, C.A.(1938). Skovtraeernes Sygdomme, Gylendal, 1, 137.
7. FREY, C.N. (1924). The Cytology and Physiology of Venturia inaequalis (Cooke) Winter. Trans. Wisc. Acad. Sci., 21, 303.
8. FUECKEL, L.(1869). Symbolae mycologicae.
9. GRAY, S.F. (1821). A Natural Arrangement of British Plants, London, 1.
10. GREEN, F.MARY.(1932). Observations on Cucurbitaria laburni (Pers.) de Not. Trans. Brit. mycol. Soc., 16, 289.
11. INGOLD, C.T. (1939). Spore Dispersal in Land Plants. Clarendon Press. Oxford.
11. (a) JOHANSEN, D.A. (1940). Plant Microtechnique. McGraw-Hill Book Co. Inc., New York and London, 189.
12. KÖCK, G. (1918). Ein für Österreich neuer Schädling auf Picea pungens. Österreich. Gartenzeitung, 13, 147.
13. LINDAU, G.(1897). Die natürlichen Pflanzen familien, Wilhelm Engelmann Leipzig, 1, 321.

14. MEHLISCH, K.(1938). Ein pilzlicher Schädling an Abies.
Blumen-u. PflBau. Ver. Die Gartenwelt, 42, 92.
15. MÜLLER, E. (1950). Eine Knospenkrankheit der Stechfichte.
Schweizerische Beiträge zur Dendrologie, 2, 69.
16. PETRAK, F. (1921). Annales Mycologici, 19, 198.
- 16 (a) TRESTON, A. and McLENNAN, E.I. (1948). The use of dyes
in culture media for distinguishing brown and
white wood-rotting fungi. Ann. Bot., (N.S.)
12, 53.
17. RIDGWAY, R. (1912). Colour Standards and Colour Nomenclature,
Ridgway, Washington, D.C.
18. SACCARDO, P.A.(1893). Sylloge Fungorum, Padua, Italy, 2, 307.,
22, 289.
19. SMITH, G.M. (1938). Cryptogamic Botany, 1, McGraw-Hill Book
Co. Ltd., New York and London, 455.
20. TUBEUF, von C.F.(1919). Schilderungen und Bilder aus norda-
merikanischen Wäldern. Naturwiss Zeitschr. für
Forst-u. Land-wirtschaft, 17, 31.
21. MASON, H.(1917). Diseases of the Common Spruce (Picea excelsa);
Observations in Darnaway Forest. Trans. of the
Royal Scottish arboricultural Soc., 31, 72.
22. WELCH, D.S.(1926). A monographic study of the genus Cucur-
bitaria in North America. Mycologia, 18, 51.
23. Anon. (1932). Report of the fifty-first Annual Conference
of the Cryptogamic Society of Scotland held on the
Benmore Estate, Kilmun, '7.
24. ROYAL METEOROLOGICAL OFFICE.(1926). Rainfall Atlas of the
British Isles, London.
- 25 (a) THE METEOROLOGICAL OFFICE. British Rainfall, H.M.S.O.,
London.
- (b) _____ Monthly Weather Report,
H.M.S.O., London.
26. THE OXFORD ATLAS.(1952). Oxford University Press, Oxford.

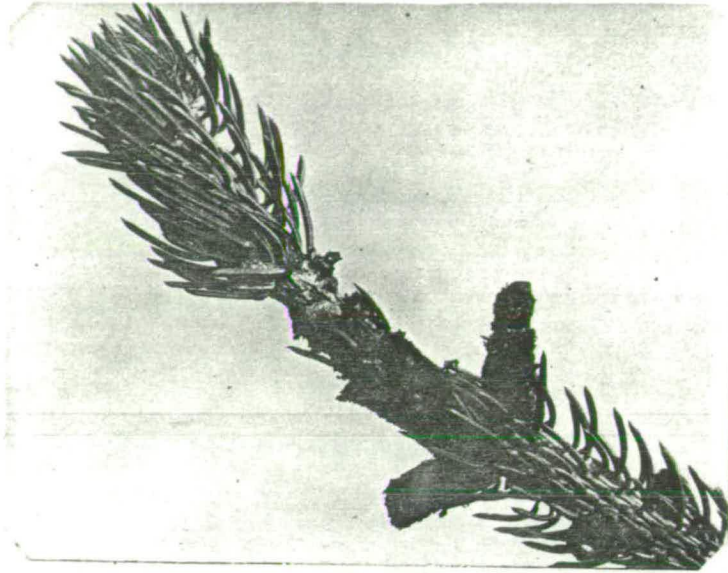
PLATE, I.

Infected branches gathered on September 21st 1950.

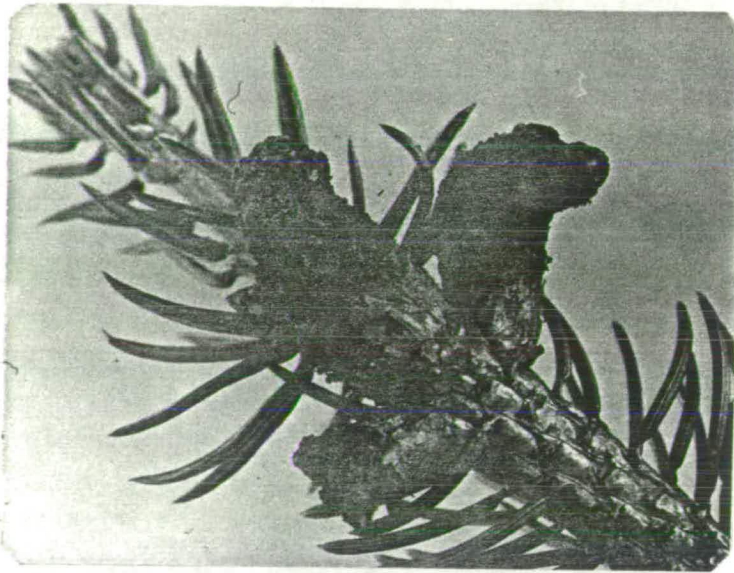
Fig. 1. The newly formed terminal and subterminal buds were infected two years previously, and in the following year; only the terminal bud formed new growth in the current year. The bud-scales formed in the previous two years bear abundant fructifications. $\times 1 \frac{7}{10}$.

Fig. 2. Distorted terminal and subterminal buds bearing fructifications. The new growth is from an uninfected subterminal bud. $\times 1 \frac{1}{2}$.

Fig. 3. The tip of a branch infected in three successive years. Few needles or leaf bases have been produced on the abnormal shoots formed each year. $\times 1 \frac{7}{10}$.



1



2



3

PLATE II.

Infected branches gathered on May 18th 1952.

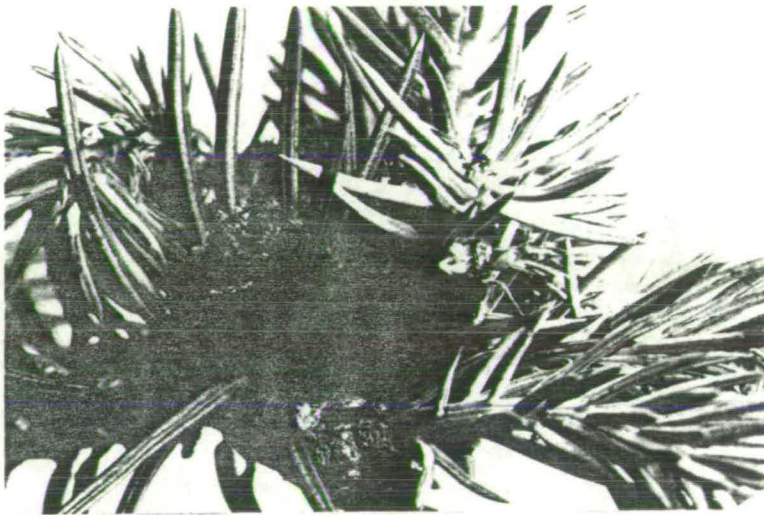
Fig. 4. Infected and distorted terminal and subterminal buds show caps of bud-scales held to the apices of the shoots by mycelium, and the undeveloped needles of the terminal shoot. The new growth is from two uninfected subterminal buds. x $1\frac{1}{2}$.

Fig. 5. Terminal and subterminal buds infected two years previously. New growth is by the eruption of shoots laterally from the infected buds which bear fructifications. x 2.

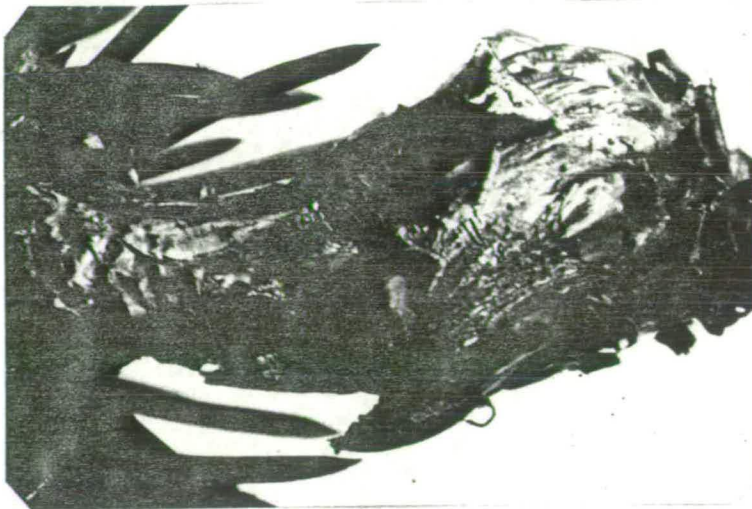
Fig. 6. The dehiscent bud-scales on an infected terminal bud bound to the base by mycelium. The deformed shoot bears undeveloped needles invested with mycelium. x $4\frac{1}{2}$.



4



5



6

PLATE III

Fig. 7. A branch showing five years of growth, and the heavy infection of all buds formed in the third year. New growth is shown at the apex of the branch and from axillary buds on five-year-old lateral branches. $\times \frac{1}{5}$.

Fig. 8. The effect of successive infections of the terminal buds is shown. A three-year-old lateral branch shows a sympodial growth pattern. The more recent growth is vigorous and entirely due to the activity of subterminal and axillary buds. $\times \frac{1}{5}$.

Fig. 9. A branch showing the partial defoliation which follows complete cessation of growth. All buds have been heavily infected and no growth has taken place for two years. $\times \frac{1}{4}$.



7



8



9

PLATE IV.

Fig. 10. Longitudinal section of an uninfected dormant bud of Picea pungens. The leaf initials of the embryo shoot are protected by bud-scales borne on a ring of primary cortex round the base(a) x 11.

Fig. 11. Longitudinal section of an infected two-year-old bud containing a constricted shoot surrounded by dense mycelium. The rudimentary needles have been broken down and the cork layer of the young shoot has become folded(c). The apical bud is protected by a few bud-scales, and empty pycnidia are borne laterally on the dehiscent bud-scales of the original bud. x 11.

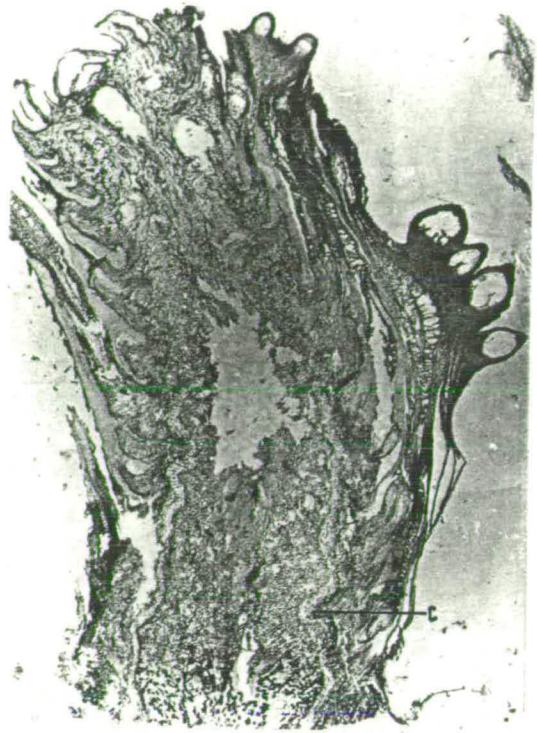
Fig. 12. Transverse section of an uninfected dormant bud showing the normal extent of the cortex and the arrangement of the bud-scales. x 13.

Fig. 13. Transverse section of an infected two-year-old bud showing the formation of a tangential cork layer across the cortex as a barrier to further penetration of the hyphae(c). The young needles have been largely broken down causing the characteristic segmentation of the pseudoparenchyma, which fills all the available space within the limits of the outermost bud-scales. x 13.

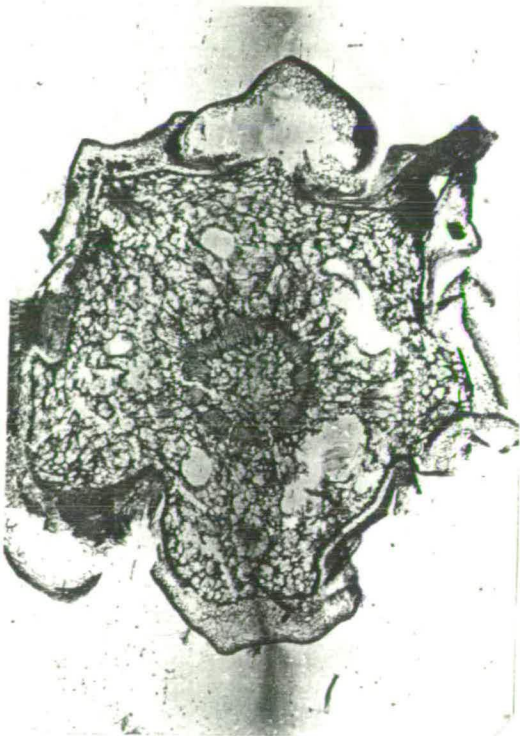
The buds shown in Figs. 10, 11, 12, and 13 were gathered at the same time in the autumn. Those in Figs. 12 and 13 were sectioned at the same point and are comparable.



10



11



12



13

PLATE V.

Fig. 14. Longitudinal section of a lightly infected bud picked on May 13th 1952 and showing an early stage in the elongation of the shoot, which has begun to twist due to the mycelium binding the bud-scales and filling the spaces within the bud. A young fructification can be seen. x 6½.

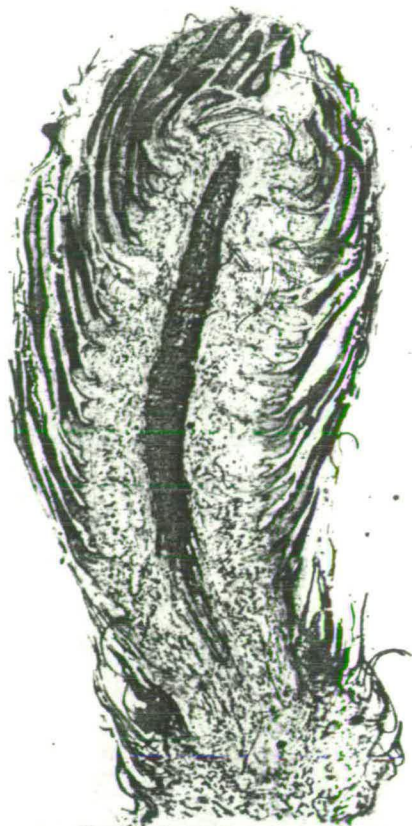
Fig. 15. Longitudinal section of a part of a heavily infected bud picked on June 11th 1952 and showing breakdown and distortion of the tissues. Young fructifications can be seen on an abortive shoot formed from a subterminal bud. x 16.

Fig. 16. Surface view of mature fructifications on a three-year-old infected bud.

a) Mature ascospores discharged under moist conditions and massed at the apices of perithecia.

b) Mature pycnidia. x 70.

Fig. 17. The fructifications shown in Fig. 16, five days later. Masses of pycnospores discharged under moist conditions and massed at the apices of pycnidia. x 30.



14



15



16



17

PLATE VI.

Fig. 18. Longitudinal section of perithecium containing maturing
asci. The infected/^{bud}was gathered on April 20th and fixed
after incubation for ten days. x 100.

Fig. 19. Longitudinal section of developing ostiole showing the
small-celled hyphae filling the cavity at the apex of the
perithecium. The material was gathered on April 20th.
x 240.

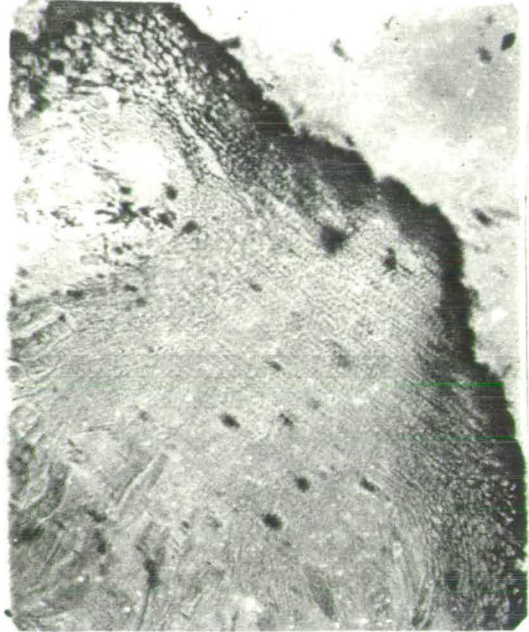
Fig. 20. Longitudinal section of pycnidium containing almost mature
pynospores and showing the sterile area at the apex and the
development of the pore.
The material was gathered on August 27th. x 100.

Fig. 21. Longitudinal section of a mature pore of an empty pycnidium
showing the periphyses meeting at the base of the canal, to
form a network.
The material was gathered on May 18th. x 460.

Figures 18 and 20 show the cells of the outermost bud-
scales adhering to the base following eruption of the fruct-
ifications.



18



19



20



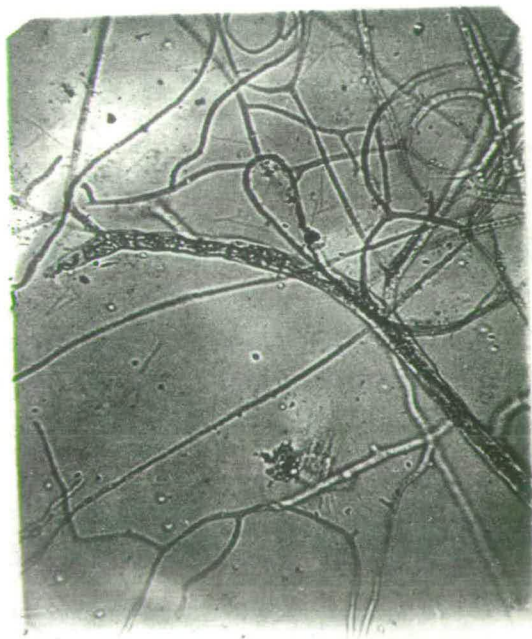
21



22



23



24

PLATE VII.

Fig. 22. The contents of single pycnidium being discharged into a drop of water; the manner in which the pycnospores are discharged from the pycnidium is shown. x 70.

Fig. 23. Side view of a pycnidium showing a tendril formed at an early stage in the discharge of pycnospores. In a dry atmosphere, the pycnospores form a mass at the pore as discharge continues. x 30.

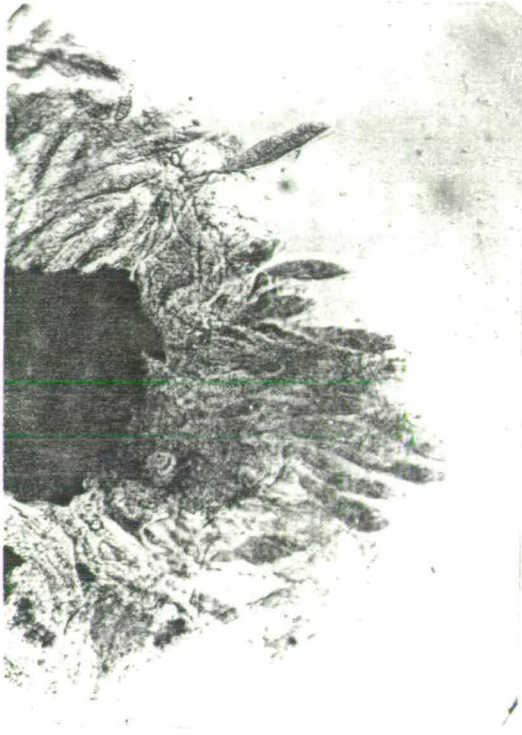
Fig. 24. A germinating pycnospore showing the germ tubes formed after 18 hours on pure agar at 18°C. x 500.

PLATE VIII.

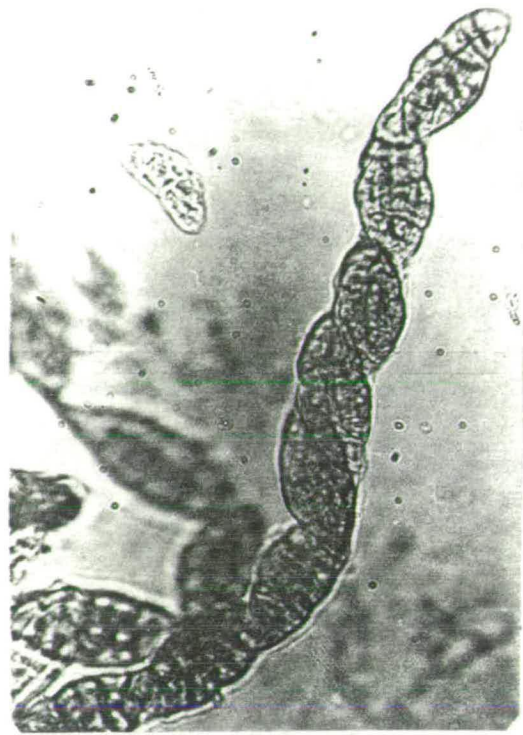
Fig. 25. The contents of a crushed peritheciium showing asci in all stages of development. x 145.

Fig. 26. A mature ascus prior to discharging spores. x 565.

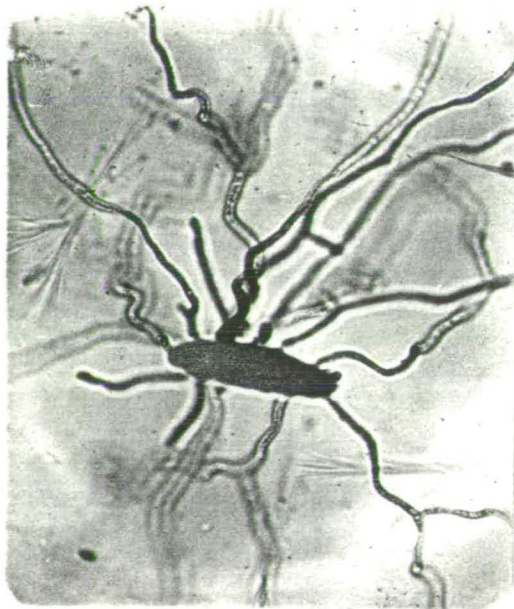
Fig. 27. A germinating ascospore showing the germ tubes formed after 18 hours on pure agar at 18°C. x 300.



25



26



27

PLATE IX.

Fig. 28. Upper surface of one month old colonies grown on malt extract agar at 5°C.

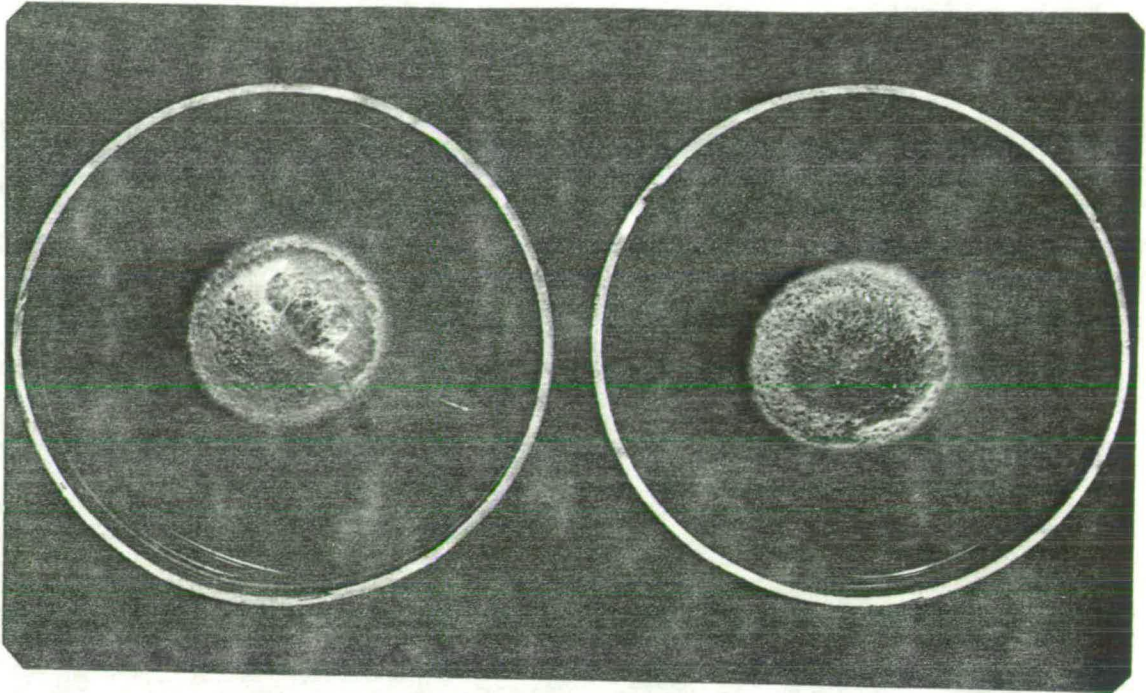
Left. Pycnospore colony.

Right. Ascospore colony.

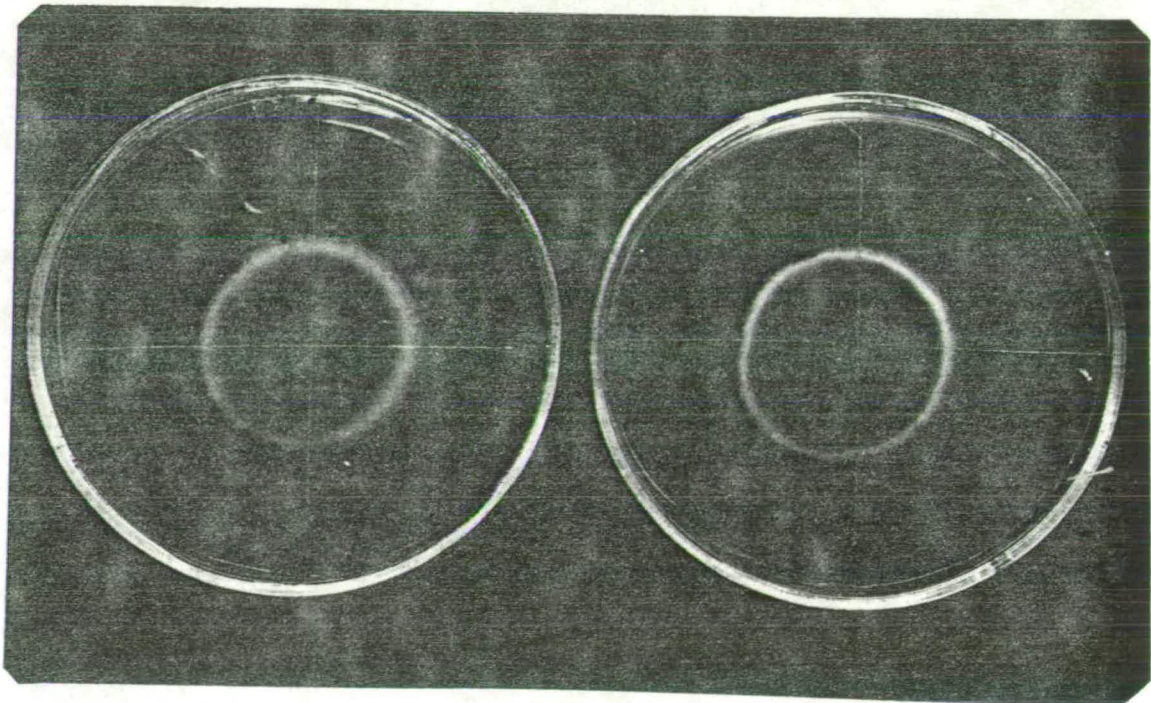
Fig. 29. Lower surface of one month old colonies grown on malt extract agar at 5°C.

Left. Pycnospore colony.

Right. Ascospore colony.



28



29

PLATE X.

Fig. 30. Upper surface of one month old colonies grown on potato dextrose agar at 5°C.

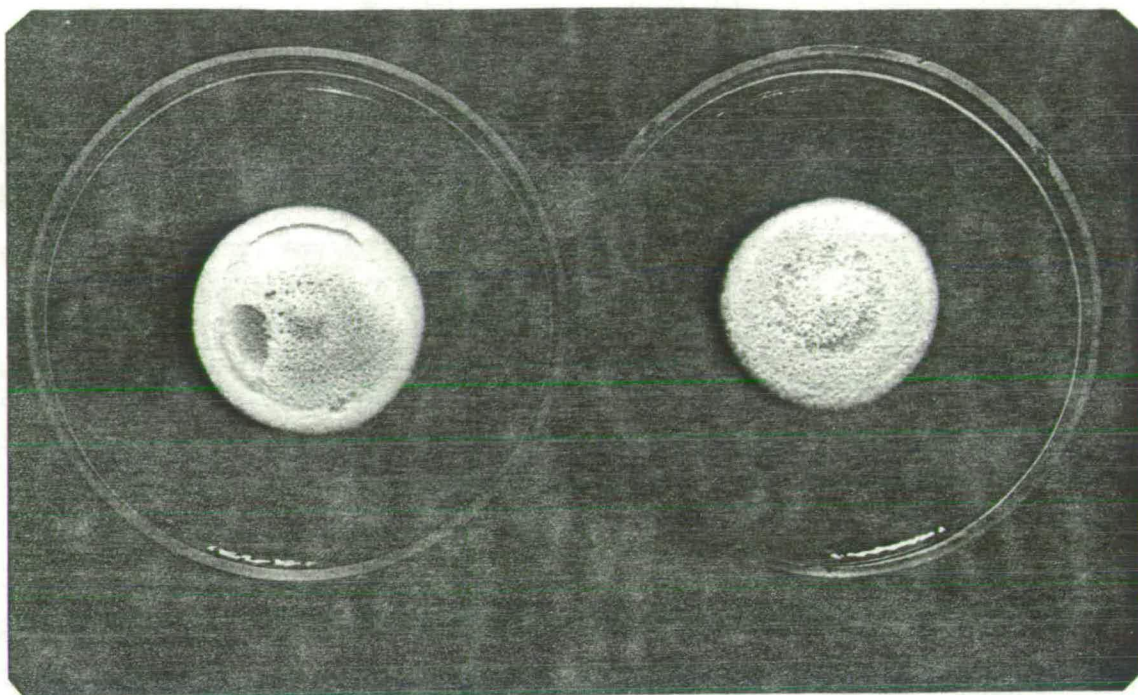
Left. Tychospore colony.

Right. Ascospore colony.

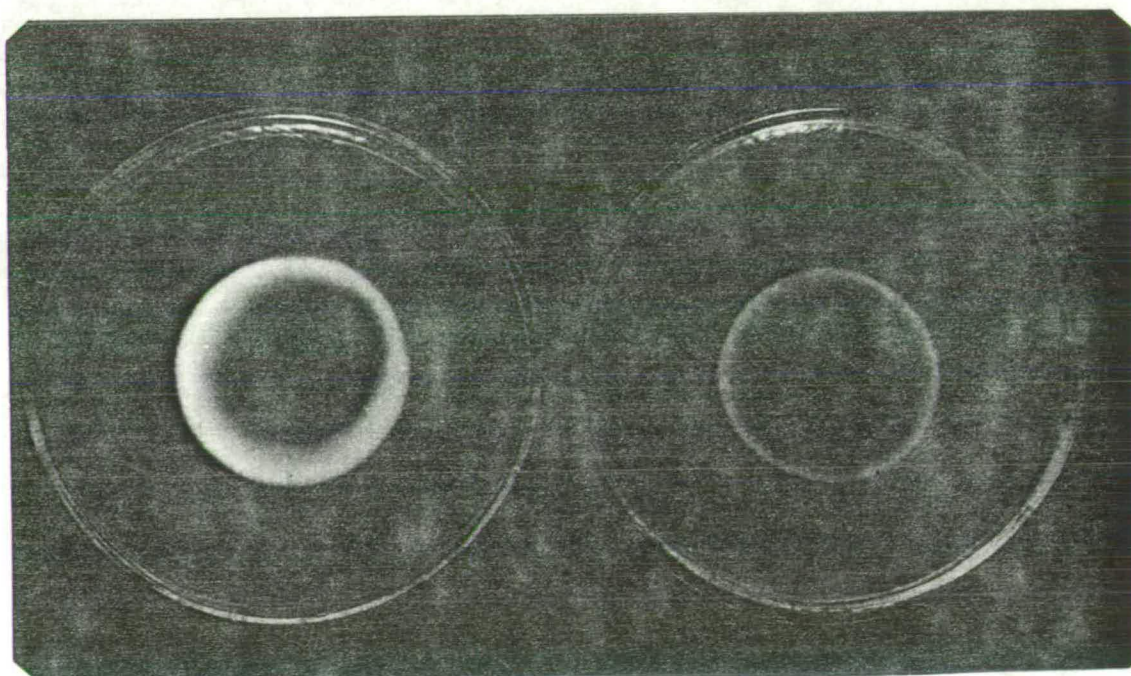
Fig. 31. Lower surface of one month old colonies grown on potato dextrose agar at 5°C.

Left. Tychospore colony.

Right. Ascospore colony.



30



31

PLATE XI.

Fig. 32. Upper surface of one month old colonies grown
on malt extract agar at 18°C.

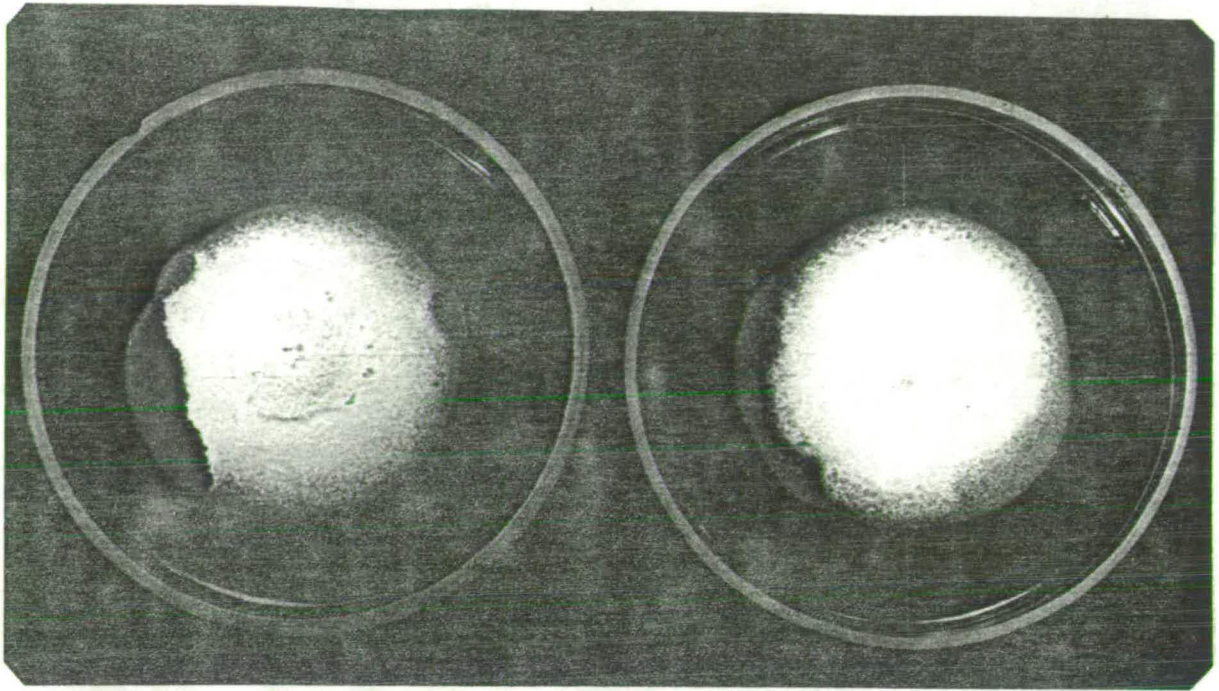
Left. Pycnospore colonies.

Right. Ascospore colonies.

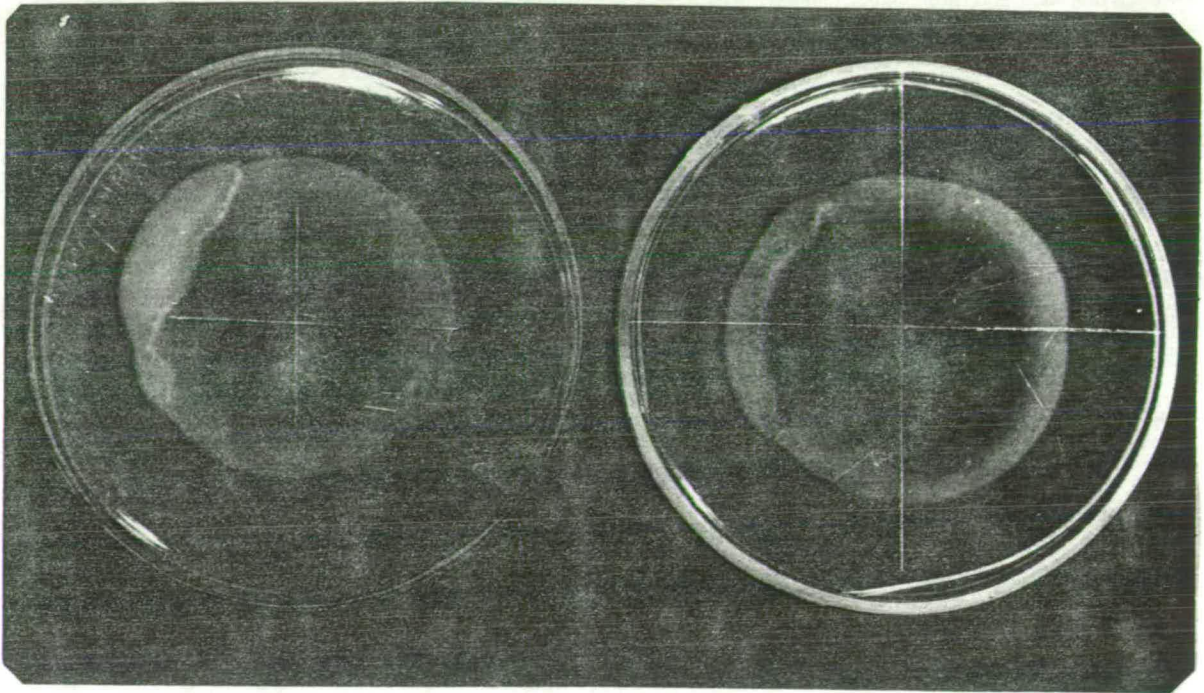
Fig. 33. Lower surface of one month old colonies grown
on malt extract agar at 18°C.

Left. Pycnospore colonies.

Right. Ascospore colonies.



32



33

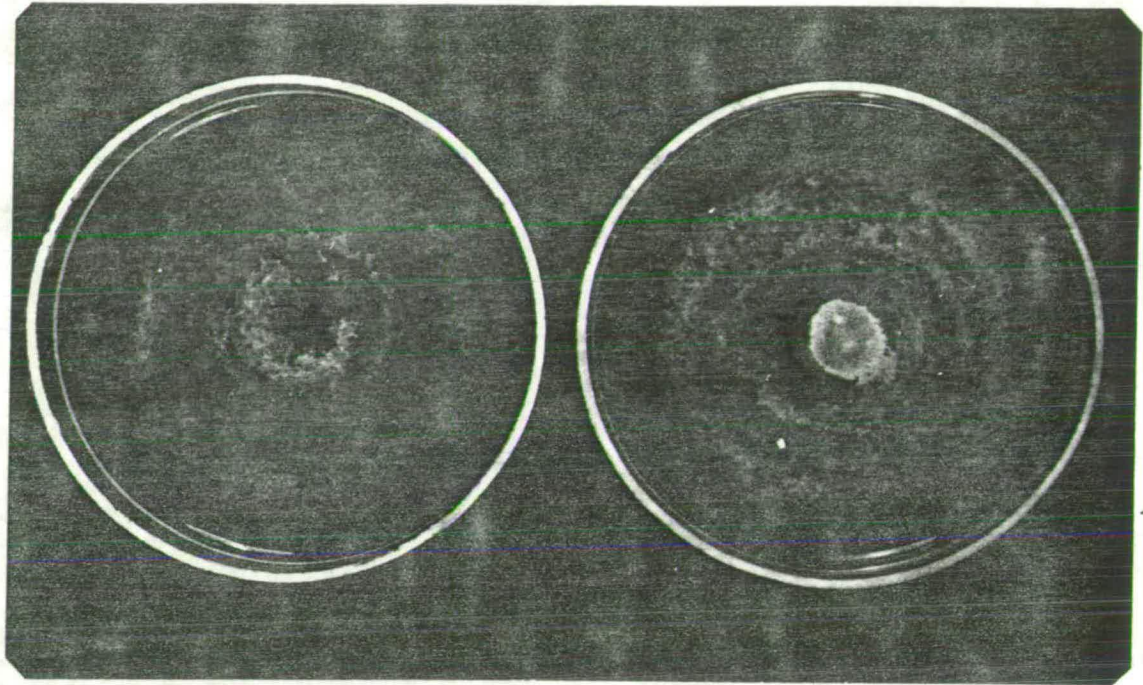


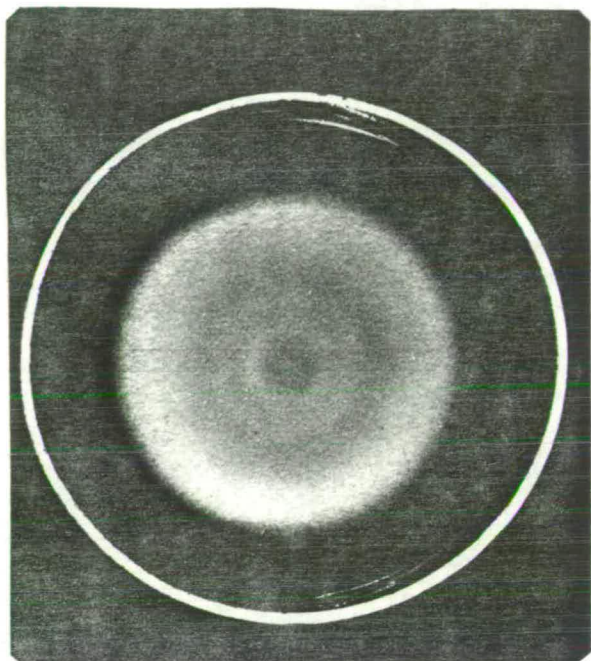
PLATE XIII.

Fig. 35. Upper surface of a one month old pycnospore colony grown on malt extract agar at 23°C.

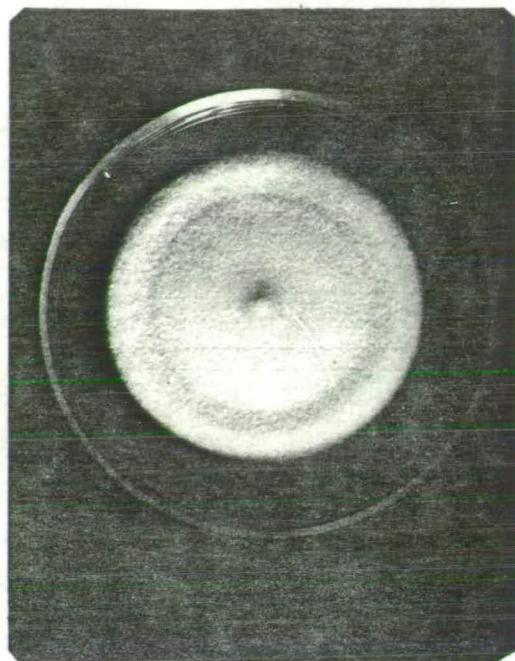
Fig. 36. Upper surface of a one month old ascospore colony grown on malt extract agar at 23°C.

Fig. 37. Lower surface of a one month old pycnospore colony grown on malt extract agar at 23°C.

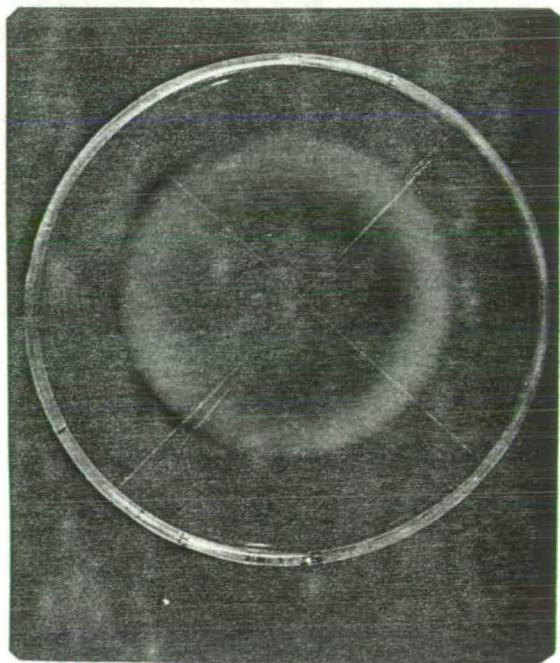
Fig. 38. Lower surface of a one month old ascospore colony grown on malt extract agar at 23°C.



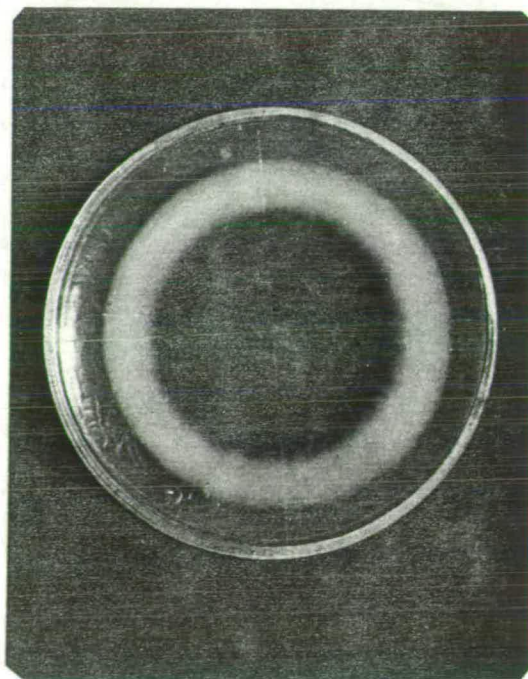
35



36



37



38

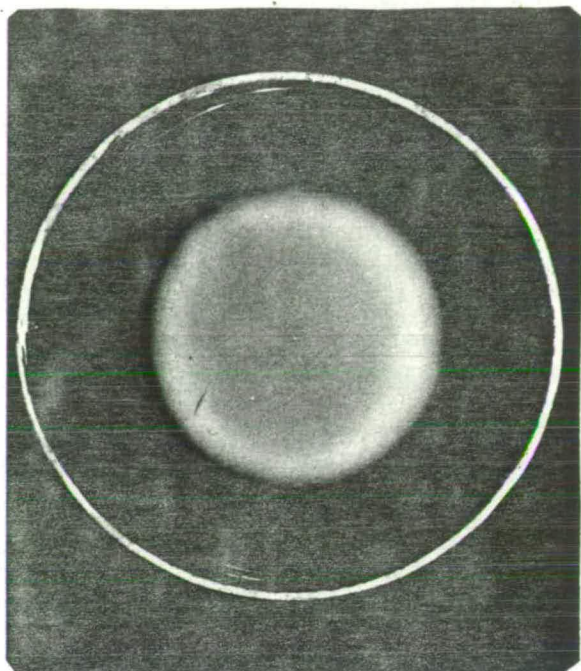
PLATE XIV.

Fig. 39. Upper surface of a one month old pycnospore colony grown on potato dextrose agar at 23°C.

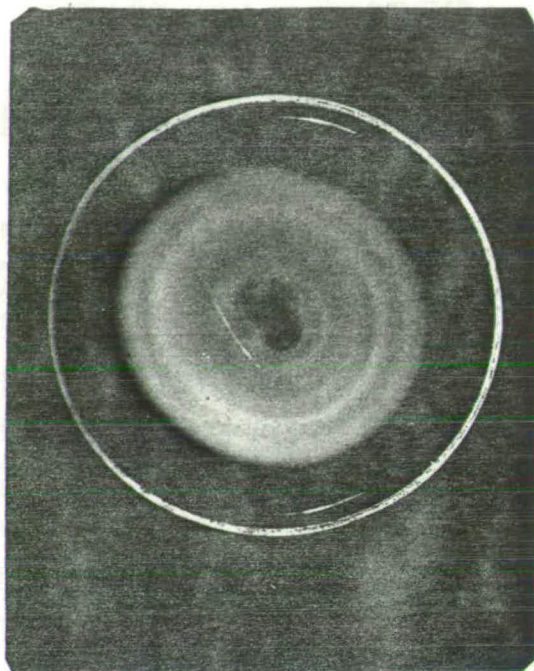
Fig. 40. Upper surface of a one month old ascospore colony grown on potato dextrose agar at 23°C.

Fig. 41. Lower surface of a one month old pycnospore colony grown on potato dextrose agar at 23°C.

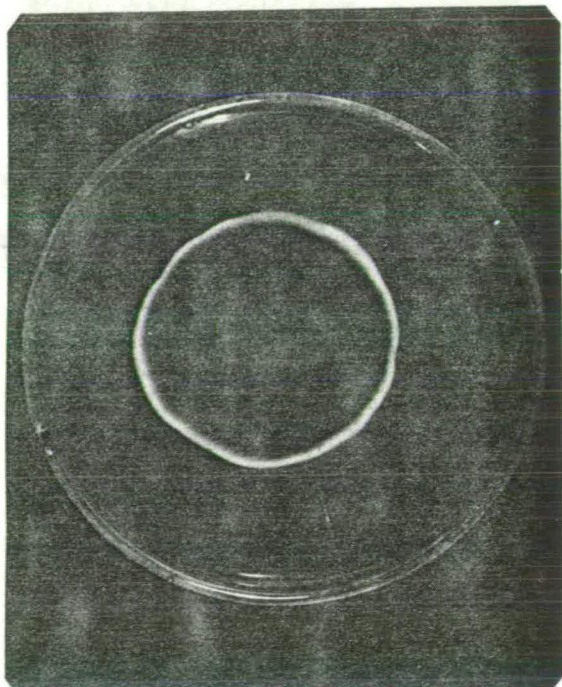
Fig. 42. Lower surface of a one month old ascospore colony grown on potato dextrose agar at 23°C.



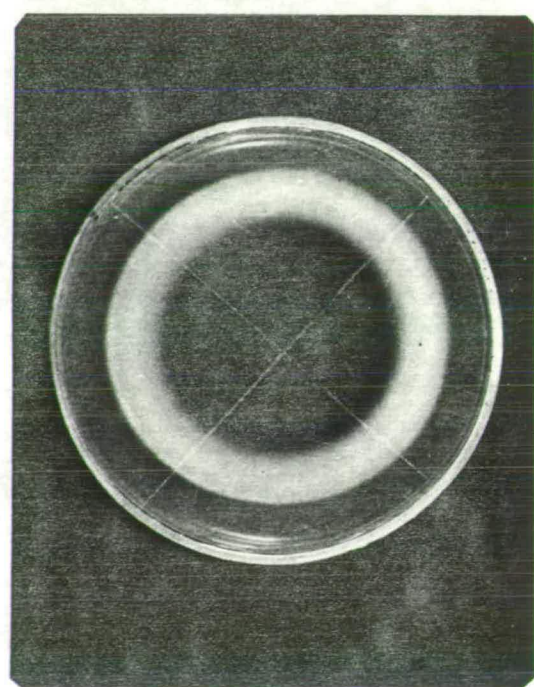
39



40



41



42

PART II.

A study of Pseudopeziza ribis Kleb.

(Gloeosporium ribis (Lib.) Mont.

and Desm.)

First of all...

Second infection.

CONTENTS.

Page

INTRODUCTION.

THE DISEASE. 5

The Imperfect Stage. 6

The Perfect Stage. 7

EXPERIMENTAL.

FIELD SITE.

PERENNATION. 8

Laboratory experiments on the discharge and
germination of ascospores.

The effect of weather on spore discharge. ... 12

Overwintering of conidia. 13

LABORATORY INFECTION EXPERIMENTS. 15

Ascospores.

Conidia. 16

Penetration. 17

Development of lesions. 18

FIELD OBSERVATIONS. 19

Primary infection.

	Page
<u>Secondary infection.</u>	20
<u>FIELD EXPERIMENTS.</u>	
<u>Perennation and infection, 1953.</u>	
<u>Objects.</u>	
<u>Lay-out of site.</u>	21
<u>Method.</u>	
<u>Results.</u>	22
<u>Effects of mulching on rate of spread of</u> <u>of disease, 1954.</u>	24
<u>Object.</u>	
<u>Lay-out of site.</u>	25
<u>Method.</u>	
<u>Results.</u>	26
<u>Fungicide tests on overwintered leaves.</u> ...	27
<u>Method.</u>	
<u>Materials.</u>	29
<u>Results.</u>	
<u>DISCUSSION.</u>	33
<u>SUMMARY.</u>	39
<u>REFERENCES.</u>	42

INTRODUCTION.

Today, there are almost three times as many acres in the United Kingdom devoted to the cultivation of black currant bushes as there were fifty years ago, and methods of cultivation have undergone radical changes during the intervening period, as a result of research into the many problems involved.

The presence in a small area of large numbers of plants of one species provides unnaturally favourable conditions for the establishment of diseases, and their control has therefore been the subject of considerable study. In addition, work on manurial treatments, cultural methods, varietal characteristics and breeding has shown that all these may play an important part in the relationship between a parasitic fungus and its host (3).

Premature defoliation of black currant bushes has frequently been the cause of considerable loss in the weight of fruit produced. This was the result of infection by Pseudopeziza ribis Kleb. (Gloeosporium ribis. (Lib.) Mont. and Desm.) a fungus causing Leaf Spot on black currants, red currants and gooseberries. Attention was drawn to its importance in this country by Britton-Jones in 1924 when, as a result of an exceptionally wet summer and early defoliation, the fruit shrivelled and the number of fruit buds was reduced (4). He showed by experiment that the disease could be considerably reduced

in even the most susceptible varieties by increasing the vigour. This was particularly evident with the variety Baldwin, which grows weakly but bears a heavy crop and is generally considered to be very susceptible. When heavily pruned in order to remove much of the fruiting wood, the vegetative growth was encouraged and the bushes suffered less exhaustion due to fruiting. On these bushes Leaf Spot was reduced to the level of less susceptible varieties.

Further work on this aspect of disease resistance was carried out by Marsh and Maynard in 1928-29 (8). These workers showed that the severity of infection varied inversely with the severity of pruning, and that effective control by this means was uneconomic, as the loss of crop was too great. Observations also showed that some reduction in the severity of infection resulted from heavy manuring of Baldwin bushes, but that this alone was insufficient to avoid loss. A combination of heavy manuring and fairly hard pruning was therefore considered advisable, in conjunction with the application of fungicides, for the control of Leaf Spot.

The fungicide recommended was Bordeaux mixture applied immediately after the crop had been picked. It was found that this arrested the progress of the disease, allowing leaf fall to take place at the normal time. The period between picking and leaf fall is of the utmost importance to the black currant bush, since it is during this time that food reserves for the following year are accumulated. The extent of the damage done by premature defoliation was shown by the fact that fruit buds from treated bushes were longer, broader and 15.3% heavier than those from untreated bushes. In the following year the treated bushes yielded 26% more fruit than the untreated ones.

It was also found that the application of Bordeaux mixture at the time of control of the disease was more effective than the application of the same mixture at a later date.

It was thought possible that the application of lime sulphur in the spring, for the control of black currant mite, would also have an effect on Leaf Spot. Marsh and Maynard found no evidence of control by this means, but Blodgett later stated that some degree of control could be exercised if the time of application was correct (2). The importance of correct timing was also found by Suit (12) who recommended the application of Bordeaux about three weeks after blossoming, or as soon as the first lesions appeared. The efficiency of Bordeaux mixture in the control of Leaf Spot has led many workers to suggest spray programmes with several applications during June and July. The use of sprays at this time has been shown to give excellent control, but the problem of residues has since led to the elimination of fungicides containing copper or sulphur. Black currants intended for processing should retain no residue, since a trace of copper catalyses the oxidation of vitamin C, and a trace of sulphide sulphur leads to corrosion of the containers and associated effects in the canning process.

With these problems in mind, Marsh and Dickinson (7) found that the application of ferric dimethyldithiocarbamate preparations fifteen days before picking gave satisfactory control of Leaf Spot, and led to no serious effects in the canning process provided that the fruit was reduced to a pulp by boiling for ten minutes before canning. Later trials of a number of fungicides of the thiocarbamate type showed them all to be effective in controlling Leaf Spot, but to be unsuitable for use on fruit to be canned whole as dessert fruit. The only spray which gave effective control and no undesirable effects in canning was heptadecyl glyoxalidine (1).

In this country therefore, research into methods of control of this disease by sprays centres mainly round those materials that leave

no objectionable residues on the fruit. Research in other countries has led to many variations in the spray materials used and the times of application, according to the local conditions encountered.

The effects of heavy manuring on the severity of Leaf Spot have already been mentioned. Later work on nutrition showed that black currant bushes have a high nitrogen requirement. Deficiencies of nitrogen and phosphorus led to an increase, while potassium deficiency led to a reduction in the severity of Leaf Spot (14). It was also shown by sand-culture experiments (13) that omission of nitrogen and phosphorus hastened defoliation and reduced the crop weights, whilst the omission of phosphorus alone had no significant effect on yield. Omission of potassium restricted growth and resulted in leaf-scorch from June onwards, while potassium deficiency led to serious reduction in the yield.

In the trials of existing varieties and the breeding of new ones, considerable resistance to Leaf Spot has been reported for a number of varieties. The older varieties Davison's Eight and Edina are regarded as being less susceptible than Seabrook's Black, Boskoop Giant and Baldwin, and the breeding of new varieties has shown that the degree of susceptibility can be altered to some extent. Cotswold Cross for example, is more vigorous in growth, and is less susceptible to Leaf Spot than Baldwin, which was one of the parent varieties (11). Other new varieties show similar tendencies while some show even greater susceptibility than older varieties.

Efforts to improve the yield and quality of black currant bushes in this country have shown that much can be done by breeding, cultivation and the use of suitable sprays to protect the bushes from the devastating effects of Leaf Spot experienced fifty years ago.

THE DISEASE.

The imperfect stage of the Leaf Spot fungus, Gloeosporium ribis (Lib.) Mont. and Desm. has long been almost universally familiar on the leaves of species of Ribes. The perfect stage was discovered by Klebahn in 1906, and named Pseudopeziza ribis Kleb. This stage was first found in the U.S.A. by Blodgett in 1932, on the overwintered leaves of wild black currant, and his specimens were considered to be the only ones collected in that country (2). According to Wormald (16) the perfect stage was recorded in this country in 1930.

As a result of Blodgett's work something is known of the cultural characteristics, temperature relationships and processes of infection of this fungus in the U.S.A. The perfect stage, however, was too rare to allow any work to be done on the perennation of the fungus on black currant leaves. In this country no attention has been paid to the presence of a probable source of inoculum on the overwintered leaves, which has been accepted as an unpleasant fact and research has been aimed solely at control of the disease during the summer months.

In early summer small dark circular spots appear on the older leaves near the base of the bush. The spots enlarge slowly becoming dark brown above and paler brown below, and sharply delimited and

angular. Several acervuli may form on either or both surfaces of each, those on the upper surface forming glistening, mucilaginous masses of spores in wet weather. As the season advances the lesions become increasingly numerous, often killing large areas of the leaves which shrivel and fall prematurely. In a wet summer defoliation may occur before the end of June, some three months early.

It has also been reported that the fungus causes golden-brown lesions on young stems, cankers on petioles and pedicels and black specks causing shelling of the fruit (5, 10).

The Imperfect Stage.

The acervuli which form during the parasitic phase arise from a basal stroma in the centre of a lesion. The conidiophores are irregularly globose at the base and have slender projections on which terminal conidia are borne. The conidia are unicellular, hyaline, curved and pointed, often more at one end than the other, and contain a number of oil drops. The spores measure $12.5 - 21 \mu \times 3.5 - 6 \mu$ (mostly $18 \mu \times 4.5 \mu$). The mass of spores eventually ruptures the epidermis, and the gelatinous matrix in which they are embedded dissolves in water, liberating the spores. New lesions appear ten to fourteen days after the establishment of infections.

The Perfect Stage.

The apothecia of Pseudopeziza ribis appear in the spring on the lower surfaces of overwintered leaves as small, whitish, fleshy structures, round and somewhat flattened on top. These rupture the epidermis exposing the hymenium, and remain partially embedded at the base. The asci are club-shaped, some 110 μ long and 20 μ wide, and have a thickened apex resembling a plano-convex lens. The eight ascospores are arranged in the ascus in two indistinct rows; they are hyaline and ovoid and contain two conspicuous oil drops, one at each end. The average size was found to be 14 μ x 6 μ , (10 - 16 μ x 4.5 - 7 μ); the spores measured had been stained and mounted in balsam and appeared smaller than those mounted in lactophenol.

Mingled with the asci are slender paraphyses, 70 μ long and 2 μ wide, swollen at the tip to 3 μ wide. The paraphyses are simple or branched and sometimes septate.

In this phase of its life the fungus is saprophytic.

EXPERIMENTAL.

Early in 1953 an investigation was begun into the life-history of Pseudopeziza ribis, with the object of establishing the methods of perennation, and the time and the conditions under which the primary and secondary infections occur in this country.

FIELD SITE.

The variety Baldwin was chosen for this work as, not only

is it one of the most susceptible to this disease, but a plot of convenient size was available for study. The bushes were planted in the winter 1940-41 at distances of ten feet by ten feet and interplanted in one direction in the winter of 1943, so that the bushes are now three feet four inches apart in rows ten feet apart. There are approximately twelve hundred bushes in twenty-four rows running almost east to west and arranged in five blocks, each ten to twelve bushes deep.

PERENNATION.

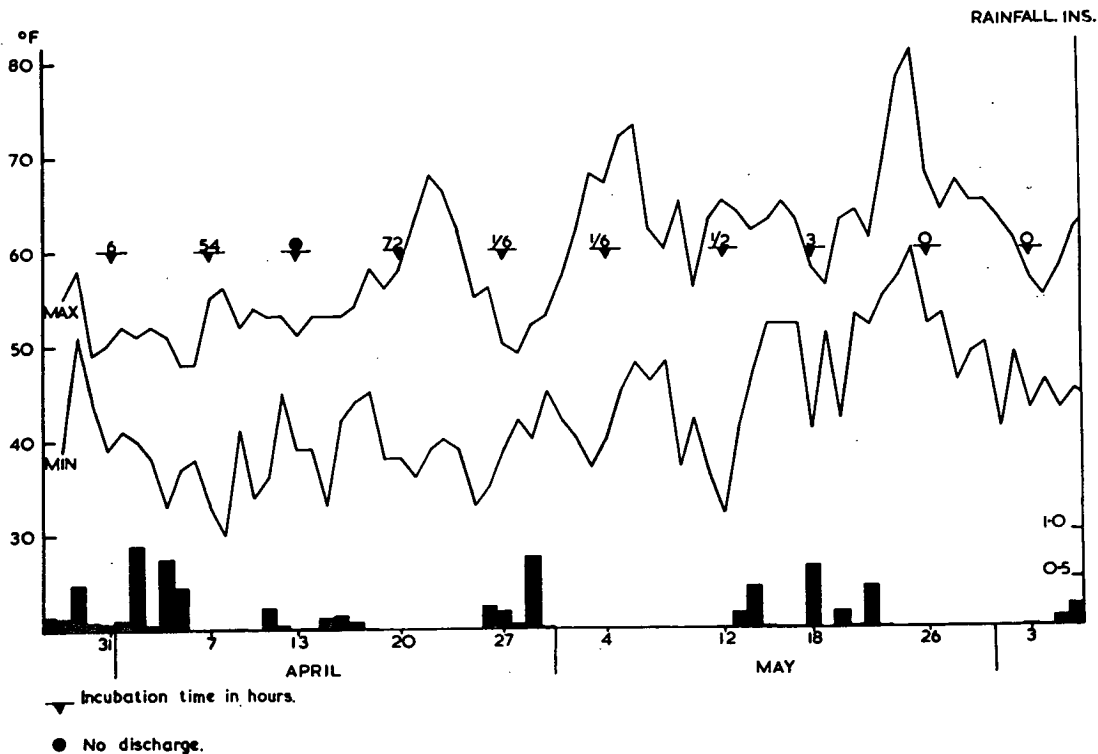
Examination of the bushes in January 1953 showed that although the majority of the dead leaves were fragmented and widely scattered over the plot, a number of whole leaves were frequently held in the stools. A number of leaves were collected on January 12th and placed in a closed chamber maintained at approximately 72°F and over 95% humidity. After fifteen days a number of small, discoid structures were observed on the lower surfaces of the leaves. Microscopic examination showed that these were apothecia containing asci and ascospores. Pieces of leaf bearing apothecia were inverted over 1.7% agar in a Petri dish but no discharge was observed.

Laboratory experiments on the discharge and germination of ascospores.

Leaves were subsequently collected at weekly intervals, placed in the moist chamber at 72°F, and removed after definite periods. A disc one centimetre in diameter was cut from an area showing apothecia and inverted over agar, and the remainder of the leaf was returned to the moist chamber for a further period. Since only one leaf disc could be tested for spore discharge each day, discs were

inverted over 1.7% agar in Petri dishes and the lids turned through forty-five degrees at regular intervals. When, on examination of the surface of the agar for spores, discharge was seen to have begun, the disc which had received the least moisture treatment and also showed discharge was transferred to a recording apparatus. The apparatus consisted of a metal cylinder, open at the top and containing a revolving clockwork drum which revolved once in twenty-four hours. The lower half of a Petri dish containing agar was placed on the top of the drum, and a piece of leaf bearing apothecia was attached to a stationary glass plate and inverted over the agar. A continuous record of spore discharge during a period of twenty-four hours was obtained by this means, and measurement of the density, or the number of spores discharged per hour from a portion of leaf of a given size was used to compare the rates of discharge under different conditions.

In 1953, ascospores were discharged from leaves gathered on March 31st and kept in the moist chamber for six hours; discharge continued for two days at laboratory temperature, but no germination took place (Text-fig. 1). No discharge occurred from leaves gathered on April 7th and kept moist for six hours but took place from leaves kept in the moist chamber for two days. Spore discharge took place from leaves collected on April 20th only after three days in the moist chamber and on this occasion the ascospores were seen to show signs of abortive germination. On April 27th ascospores were discharged from leaves moistened for ten minutes; the discharge



TEXT-FIGURE 1.

Meteorological records in relation to ascospore discharge for the period March 31st - June 5th 1953.

The figure shows the incubation time required for initiation of spore discharge from the date of collection of overwintered leaves.

The records given are for the 24-hour period from 0900 hours G.M.T. on the date given.

continued for two days and within one day the germ tubes were five times the length of the spores.

Spore discharge and germination occurred abundantly during May and on June 3rd leaves collected while still damp and inverted over agar gave immediate discharge.

After this time very few dead leaves were to be found for these experiments, so large numbers of fallen leaves were gathered in the autumn of 1953 and overwintered in trays. The trays were two feet square and six inches deep, divided into nine compartments and covered above and below with fine wire netting. The compartments were designed to minimise the movement of the leaves on windy days, and this was further reduced by placing the trays over canvas on a rack and covering them with more canvas during high winds. The trays were placed on the grass for considerable periods, but were returned to the rack in exceptionally wet periods. In this way the leaves were subjected to normal weathering without the destructive effects of wind, waterlogging and cultivation, and complete leaves were available for experimental work throughout the summer of 1954.

The discharge of ascospores was first recorded in 1954 on April 6th, after overwintered leaves had been six hours in the moist chamber. Germination occurred only after leaves were given moisture treatment lasting twenty-four hours and the discharged spores kept for two days at 76°F. By May 10th the time required in the moist chamber had fallen to thirty minutes, but the rate of spore discharge remained low except from leaves receiving moisture treatment for more than four hours. Germination occurred fairly readily at laboratory

temperature and was considerably increased by keeping the Petri dishes at 76°F for two days.

On May 18th the apothecia discharged ascospores in considerable numbers after no moisture treatment, and germination at laboratory temperature was moderately good after twenty-four hours. This was repeated on May 25th and June 2nd, but thereafter discharge was very slow and germination nil.

Examination of the rate of spore discharge revealed a tendency to diminish as daylight diminished and to increase as the light increased. Apothecia from which discharge was at best only moderate, showed no further discharge after late evening, whereas those discharging large numbers of spores showed a definite decline in the rate of discharge during the middle of the night and a return to a higher rate of discharge after dawn. Although variation in temperature might also have a depressing effect on spore discharge at night, the variation in the laboratory is relatively small.

The possible effect of light on discharge was shown by inverting two discs of leaf tissue over agar, one in the light and one in darkness. Slight discharge was observed after twenty-four hours in the light, followed by a moderate level of germination. In the dark, no discharge took place over a period of seventy-two hours.

By the above method, and using leaf discs which were wet or dry when inverted over agar in a Petri dish, it was found that if the leaf bearing apothecia was moistened without wetting the apothecia, discharge was almost immediate and plentiful. Dry leaf discs required some hours to absorb moisture from the atmosphere before discharge took place, and once wet, discharge in the relatively dry atmosphere of the laboratory was at a higher rate than in the humid atmosphere of a moist chamber.

Whatever the conditions of light and temperature, the percentage germination was low, but appeared to be higher in the light than in darkness. Attempts to increase the percentage germination were found of little value, although a temperature of 76°F. generally caused some improvement and increased the growth of germ tubes.

The effect of weather on spore discharge.

Comparison of the results obtained in two successive years in the laboratory experiments described above, indicates that spore discharge could be expected to occur in the first week in April if weather conditions were favourable for twenty-four hours or more. The required period of high temperature and moderate moisture diminish during April, and by the beginning of May only thirty minutes of favourable conditions are needed to initiate spore discharge. The peak period for ascospore discharge in 1953 appears to have been from the last few days of April to the first few days of June, whereas in 1954 the period was from mid-May to early June. In both years however, the second half of May was a time when suitable conditions induced an almost immediate discharge of spores, and a high rate of germination followed at normal temperatures.

During the period March 28th - June 5th 1953 the rainfall was spasmodic, although there was a trace almost every day except during the periods April 19th - 25th and May 1st - 11th (Text-fig. 1). Leaves collected on April 27th, two days after the end of the first dry spell, required only ten minutes in the moist chamber before spore discharge began: those collected on May 18th, however, required three hours. Dry leaves collected on May 26th, after high temperatures on the previous few days, discharged spores immediately after being moistened. Comparison of the weather records during the week prior to

collection of leaves on these three occasions seems to indicate that temperature exerts a greater influence than rainfall on maturation of the apothecia. Some moisture is nevertheless essential for spore discharge, and the effects of various conditions noted above suggest that the early morning would be the period of maximum output. Apothecia on moist leaves would then be turgid and would be stimulated to discharge spores as light intensity increased and the air became drier.

Overwintering of conidia.

Although the presence of a probable source of inoculum on the dead leaves has been generally accepted, it has been suggested that the overwintering of conidia in the buds might be an important method of perennation.

When the leaves first began to appear on the black currant bushes at the end of February 1953, a number of shoots were collected weekly and placed in water in the laboratory. No lesions were observed on any of the leaves on these shoots until June 9th, when leaf lesions became visible on shoots collected two weeks previously on May 26th. By this time the discharge of ascospores was taking place readily in the laboratory, and these lesions were in all probability the result of natural infections with ascospores. Overwintered conidia, had there been any within the buds, would have germinated and produced symptoms of the disease a long time before, unless the winter temperatures had induced a period of prolonged dormancy. In order to test this possibility, potted black currant bushes kept in isolation were examined throughout the season. No spots were observed until very late in the year and these were almost certainly caused by chance infection.

Bushes in pots buried in the soil in the untreated area of the experimental site. were severely infected during the summer, and were lifted and returned to the laboratory on November 6th. Two bushes were sprayed with a 0.3% solution of Belsan (sodium hypochlorite) with 0.3% Estol as a wetter, care being taken to cover the soil during application; after one hour the bushes were rinsed and placed outside in ashes with three untreated bushes. No spots were observed on the leaves of any of these bushes in 1954.

Other potted bushes were treated with suspensions of conidia from infected leaves before being placed outside in ashes for the winter. A warm autumn in 1953 caused a number of buds to break and leaves to open prematurely. Drops of spore suspension at a concentration of ten thousand conidia per millilitre were placed in the axils of leaves and on buds on half the bushes and the bushes placed in a damp chamber for two days at 72°F. The bushes were then removed and after drops of spore suspension had been placed on the remaining buds, were placed outside. The control bushes were given the same treatments, but no spore suspensions were placed on the buds. No lesions were seen on the leaves of any of these bushes in 1954. These experiments indicate that if any conidia overwinter in the buds, the number must be very small, too small to account for the widespread distribution of the lesions when they first appear in the early summer.

Subsequent experiments were designed to test the viability of overwintered conidia. Conidia were washed from autumn leaves and centrifuged, and some of the spore suspension was put in Petri dishes and placed in the open. The lids of the dishes were fixed in such a way that the conidia were exposed to all changes of weather during

the winter, except for rain. The rest of the suspension was put on filter paper in funnels and covered with muslin, so that the conidia were exposed to winter conditions with intermittent moisture. In the spring the conidia were sprayed on to the leaves of potted bushes and subjected to germination tests. The results were negative in both cases.

During examination of overwintered leaves for apothecia, a number of glistening masses of conidia were found. The conidia were very variable in shape and size but infection and germination tests were successful. These conidia seemed to occur on the lower surfaces of leaves on which, for some reason, there were no apothecia.

There are, therefore, two possible sources of primary infection in the spring from overwintered leaves.

LABORATORY INFECTION EXPERIMENTS.

Ascospores.

When it was found that ascospores were forcibly discharged from apothecia induced by the moist chamber treatment described above, potted black currant bushes were used for infection experiments in the laboratory. Portions of leaf bearing apothecia were attached to microscope slides fixed to strong wires pushed into the soil. The fragments of the leaf were held firmly at various distances from the undersides of leaves, and the leaves mapped to show the areas in which successful infections should appear.

On April 30th two potted bushes were placed in a moist chamber and a number of leaf fragments were arranged close to the leaves. After five days the bushes were removed from the chamber and two days later the leaf fragments were removed.

Lesions typical of Leaf Spot appeared nine days after the leaf

fragments were placed in position, and these were restricted to those areas held directly above the fragments. Acervuli which soon formed contained conidia of P. ribis. Three weeks after the infections took place a single leaf was sprayed at intervals over a period of thirty minutes with distilled water. Five millilitres of liquid were collected from the tip of the leaf and found to contain two hundred and twenty three thousand conidia per millilitre.

No infections resulted from leaf fragments allowed to discharge ascospores on to the upper surfaces of leaves, but where the fragments were held close to the lower surfaces infection occurred readily. Where the distance between the lower surface of a leaf and the apothecia was increased the frequency of infections was reduced. Fragments less than ten millimetres away gave successful infections, but beyond this distance infection was nil. In one case infection was successful when the gap between the leaf fragment and the lower surface of a leaf necessitated the ascospores being discharged horizontally a distance of four millimetres.

Conidia.

On May 22nd conidia collected in washings from a leaf infected with ascospores were sprayed on to the leaves of two potted bushes at a concentration of twenty thousand spores per millilitre. The bushes were placed in the moist chamber for one day and then kept in the laboratory; lesions were just visible after seven days.

As was found in infection experiments with ascospores, no infection resulted from spraying only the top surface of the leaves. Spraying both, or only the lower surface resulted in infections of similar density, and it appears that the only method of infection ^{by} is/germination and penetration through the lower surface of the leaf.

Penetration.

Experiments were carried out with excised leaves placed in Petri dishes to permit observation of germination, penetration and the early stages of the establishment of the fungus within the leaf. Leaves were placed on damp filter paper with the lower side facing upwards and ascospores were discharged from a leaf fragment attached to the lid of the dish. The leaves were removed, and the infected areas mounted in cotton blue in lactophenol for microscopic examination.

The formation of appressoria and branched hyphae within the epidermal cells was found after six days, although the actual time of discharge on to the leaf is uncertain. Discolouration and disorganisation of leaf cells was seen after eight days when the lesions were 0.3 mm. in diameter and just visible to the naked eye. After ten days conidia were being formed and an exposed acervulus was seen after twelve days, when the lesion was 0.5 mm. in diameter. During this experiment, notice was taken of the position of germinating ascospores, and no evidence was found of any penetration of stomata pores by this fungus. Germination in this case is followed by the direct penetration of the cuticle and the wall of the epidermal cell, after the formation of an appressorium. As Blodgett points out (2) the formation of an appressorium is sometimes omitted by germinating conidia, the spores themselves acting as appressoria. In spite of their smaller size, the ascospores also appear to act as appressoria occasionally, the germ tubes emerging below the spore and penetrating directly downwards.

The fact that penetration occurs whatever the position of the leaf surface implies that it is probably due to a haptotropic response,

and that no matter where the germ tube emerges, it is contact with the surface of the leaf which stimulates the formation of an appressorium and leads to penetration.

Development of lesions.

The infection of black currant bushes in the laboratory enabled regular observations to be made on the growth and development of individual lesions. With both ascospores and conidia the lesions were first visible after seven days as minute discoloured spots on the leaves. Acervuli formed rapidly and released conidia after twelve to fourteen days, when the maximum diameter was 0.5 mm. After twenty-one days the diameter was 1.0 mm., and after twenty-eight days 2.0 mm. Growth beyond this size is restricted to some extent by the venation of the leaf. Whereas at first the lesions develop radially in the concave areas bounded by the venules, the hyphae soon penetrate to the minor veins and expansion is restricted at these points. The whole area bounded by veins and venules is soon filled with hyphae and disorganised cells which become increasingly discoloured. The bigger the veins bounding the lesions the greater is the resistance to further penetration by the hyphae.

The characteristically angular outline of the lesions is thus a result of the polygonal arrangement of the veins of the black currant leaf, and the restriction that the veins place on expansion of the lesion. Growth beyond the area originally occupied by the fungus occurs slowly, although once penetration of a minor vein has been achieved, the death of the cells in the adjoining area is

TEXT-FIGURE, 2.

Growth of lesions formed on a black currant leaf sprayed on June 24th 1953 with a suspension of twenty thousand conidia per millilitre. The conidia were sprayed on to the lower surface of the leaf.

1. I. Appearance of the lesions after 21 days.
 II. Appearance of the lesions after 63 days.
 III. Appearance of the lesions after 167 days.

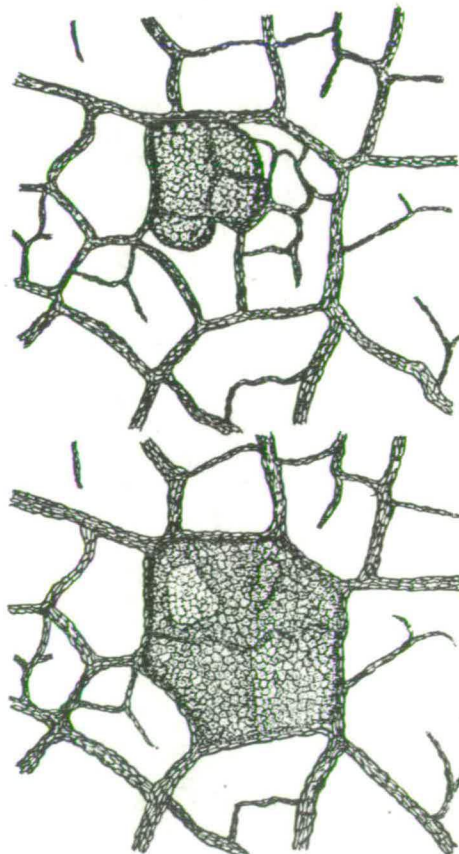
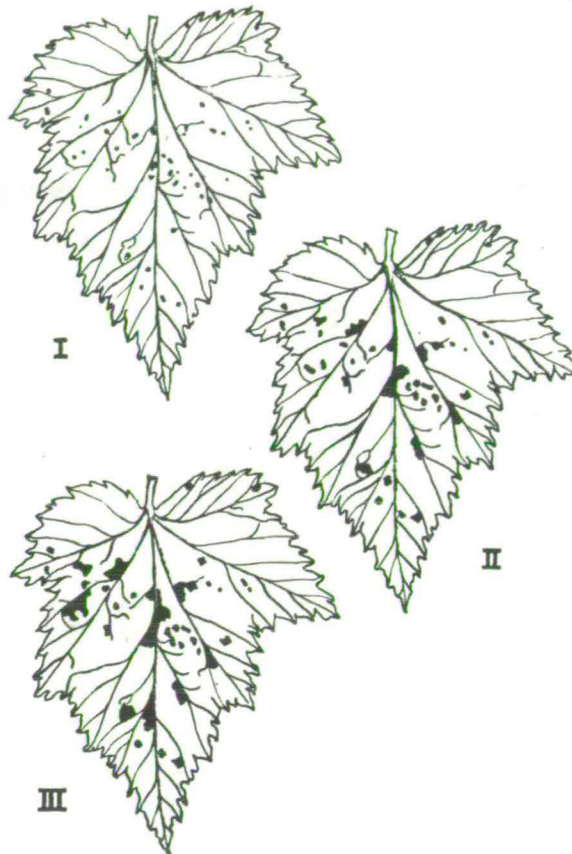
x ½

2. Growth of a single lesion..

Above - after 21 days.

Below - after 63 days.

x 45



rapid. In the absence of reinfection by water-borne conidia, the largest lesions measured 4.5 x 1.5 mm. after forty-nine days, 10 x 4 mm. after eighty-four days and 16 x 5.5 mm. after one hundred and twelve days. Such measurements were exceptional and may well have resulted from multiple infections in a small area, since the majority of lesions measured about 5 x 1.5 mm. four months after infection (Text-fig. 2).

FIELD OBSERVATIONS.

Primary infection.

When it was established that the perfect stage of this fungus could occur abundantly in this country, observations were made on primary infection in the field. The laboratory experiments on the maturation of apothecia provided early information on their stage of development in the field, enabling an estimate of the probable time of natural spore discharge to be made. As has been mentioned, some of the first lesions were seen in 1953 on leaves taken from the bushes on May 26th, while in the field lesions were found on a large number of leaves on June 3rd. These latter would have resulted from infections about May 20th, so that the last week in May would seem to have been the peak time of primary infection.

The earliest lesions were found on the lower leaves, particularly those associated with the fruit spurs. At this time the majority of the leaves on the old wood were beginning to turn red, and the fact that the lesions were mostly observed on these leaves suggested a greater susceptibility to attack. Later in the season, when older leaves were more frequently found near young leaves, infection occurred as readily on young as old.

Secondary infection.

The disease spreads rapidly from the area of primary infection, travelling distally along the branches, and reaching the most recently formed leaves about the first week in August. In order to establish the time taken for a healthy leaf to become infected and killed, a number of clean leaves were marked immediately following a heavy rainstorm. It was found that within five or six weeks, with fairly frequent rain, about four waves of infection took place, each heavier than the last. At the end of this time the leaves had withered and fallen, the damage being due to the rapid spread of infection arising from the enormous spore output from each acervulus, and the speed with which spores were formed by the second and third generations.

Towards the close of the season the acervuli on the upper leaf surfaces were found to become empty, the conidia being formed only on the lower surfaces. A large number of lesions were very dark, due to the presence of heavy dark hyphae. Microconidia were abundant at this time in the acervuli on the lower surfaces of the leaves. These were rodlike, unicellular, hyaline bodies with shortly rounded ends. The probability of their function, if any, being of a sexual nature is supported by their formation late in the season on the abaxial surface, that on which the apothecia develop.

FIELD EXPERIMENTS.

Perennation and Infection, 1953.

Objects.

In addition to laboratory experiments on perennation and

infection a field experiment on these subjects was carried out in 1953. The objects were to establish the time of natural infection, the rate of spread and defoliation, and to test the possibilities of control by attacking the fungus on the dead leaves.

Lay-out of site.

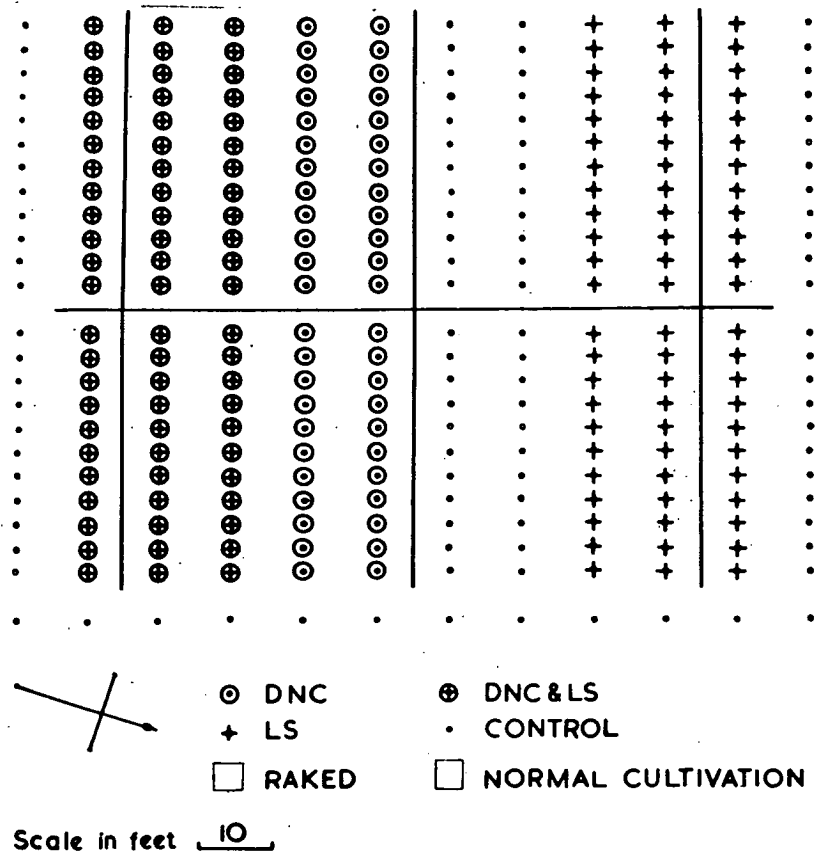
Two hundred and thirty bushes of the variety Baldwin in ten rows were divided into four parts each consisting of five rows of eleven or twelve bushes. The two squares on the western side received one treatment, those on the eastern side another, one of each pair on the southern side another and the second square of each pair another. Superimposed on two rows of each one of the four original squares was yet another treatment, giving a total of eight in all (Text-fig. 3).

Method. The treatments were as follows:-

Raking. Dead leaves and fragments were removed by hand and by raking from half the bushes, the two western squares, on February 12th.

Spraying.

(a) A 0.1% solution of dinitro-ortho-cresol in 2% oil emulsion was used as a ground spray on half the bushes, the two southern squares, on February 25th. The spray was applied with a twin-nozzle hand lance at a pressure of seventy-five pounds per square inch at a rate of six hundred and fifty gallons per acre. The soil was given a liberal covering of spray, which was also applied to the stools to a height of six inches from the ground.



TEXT-FIGURE 3.

Perennation and infection, 1953.

Arrangement of treatments on experimental site.

(b) 1½% lime sulphur was applied to the two innermost rows of all four squares on April 8th.

The remainder of the site received normal cultivation during the summer, but in order to leave the surface of the treated area undisturbed, a heavy mulch of partly-composted straw was applied to the whole area in mid-February.

Assessment of the amount of Leaf Spot was made by a count of the number of leaves showing lesions on marked bushes selected at random. The first count was made on June 3rd when the symptoms had only just appeared, and on this occasion the total number of infected leaves was counted. This figure was expressed as a percentage of the total number of leaves per bush, an arbitrary figure of one thousand being taken for this purpose. On June 29th and July 21st counts were made of the numbers of infected and uninfected leaves on five shoots selected at random from each marked bush.

The outermost treated row on each side of the experimental plot was regarded as a guard row and omitted from the assessments. The crop was picked after July 16th.

Results.

Table 1.

Treatment.	<u>PERCENTAGE INFECTION</u>					
	June 3rd	Mean	June 29th	Mean	July 21st	Mean
Control	16.05	16.05	61.00	61.00	94.91	94.91
LS	6.25)		50.74)		94.91)	
DNC	6.20)	4.90	45.67)	43.58	97.75)	94.54
Raking	2.25)		34.33)		90.96)	
DNC+LS	1.35)		19.86)		89.93)	
Raking+LS	0.95)	1.07	24.07)	23.91	86.91)	87.35
Raking+DNC	0.90)		27.81)		85.21)	
Raking +DNC+LS	0	0	10.99	10.99	52.01	52.01
LS. Lime sulphur. DNC. Dinitro- <u>ortho</u> -cresol.						

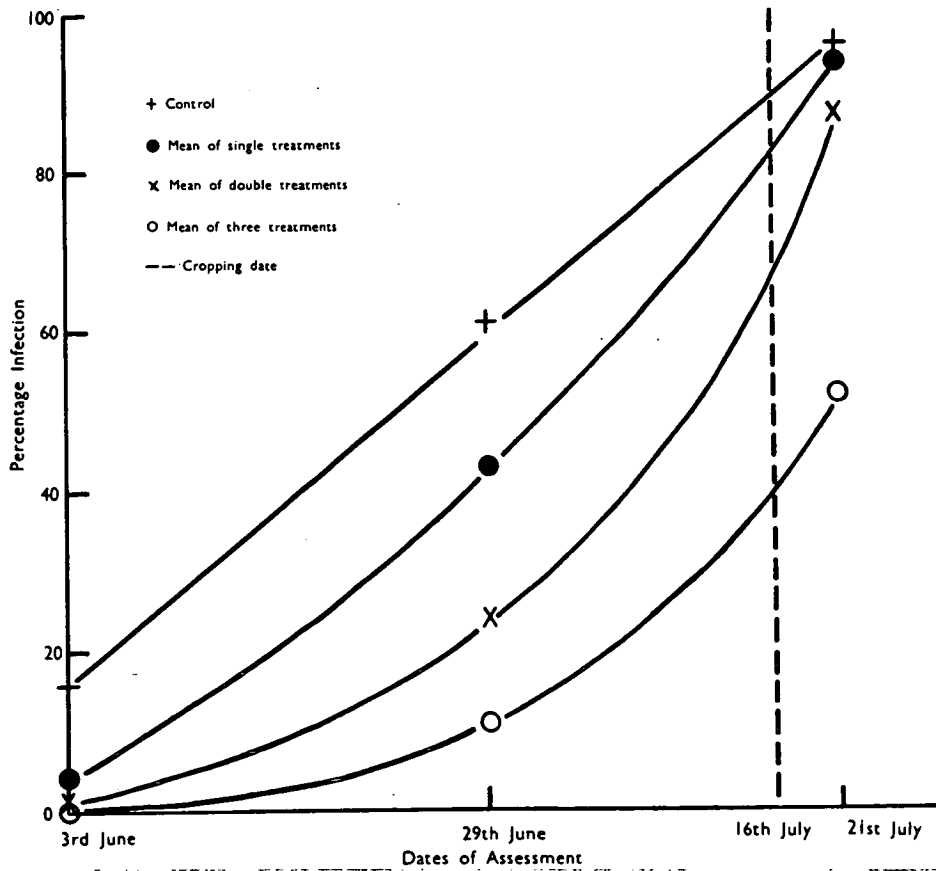
Statistical analysis of the results given in Table 1 showed that each of the three treatments had a significant overall effect on the incidence of Leaf Spot, an effect which persisted beyond the cropping date. The analysis also showed that although raking was consistently the best single treatment, no one treatment or pair of treatments was significantly better than another. A combination of all three treatments, however, remained significantly superior to all other treatments throughout the season (Text-fig. 4).

Three weeks after the last assessment of percentage infection, practically all the leaves were infected and defoliation was also well advanced, especially on the untreated portion of the site. Two more assessments were made, on August 25th and September 22nd the percentage defoliation being estimated on every bush as a whole.

Table 2.

<u>Treatment.</u>	<u>PERCENTAGE DEFOLIATION</u>			
	August 25th.	Mean.	September 22nd.	Mean.
Control	89.10	89.10	99.10	99.10
LS DNC Raking	85.91) 87.28, 72.92)	82.0	93.60) 95.45) 95.85)	94.96
DNC+LS Raking+LS Raking+DNC	70.51) 67.92) 66.26)	68.23	91.35) 91.25) 90.00)	90.86
Raking+DNC + LS	70.42	70.42	86.25	86.25
LS. Lime sulphur. DNC. Dinitro- <u>ortho</u> -cresol.				

In view of the heavy losses of leaves suffered during the picking of the fruit, no statistical analysis of these results was carried out. The same trends as were noticed earlier in the season were again found here, but the advantages of one or



COMPARISON OF TREATMENTS.

more treatments over control became less marked as the autumn advanced. This would be expected from Text-fig. 4 which shows that the differences between the treatments and control, based on percentage infection of the leaves, diminished rapidly after the cropping date. Defoliation in August and September is almost entirely the result of Leaf Spot infections, and percentage defoliation therefore reflects the course of the disease after percentage infection has reached its maximum. The speed with which the disease spreads gives little time for more than a few assessments based on either criterion, and the results must be judged separately until some method is devised for assessing the progress of this disease throughout the entire season.

Effects of mulching on rate of spread of disease, 1954.

Object.

The mulch applied to the treated area in 1953 appeared to have a retarding effect on the primary infection when the control bushes were compared with the untreated remainder. Apart from the advantages of moisture conservation, hoeing becomes unnecessary when a mulch is used, and leaf fragments buried in the soil remain undisturbed. Mechanical cultivation has disadvantages in that the use of a tractor-drawn tined cultivator leads to the formation of deep wheel tracks close to the bushes, while the cultivator merely breaks up the surface and leaves the soil in lumps. Splashing of cultivated soil during summer rainstorms carries mud to a height of three feet or more, and it is probable that much of the spread of conidia is due to this factor. In 1954, therefore, the effect of mulching with straw between the bushes was studied, the object being to reduce the amount of disturbance of the soil and the amount of

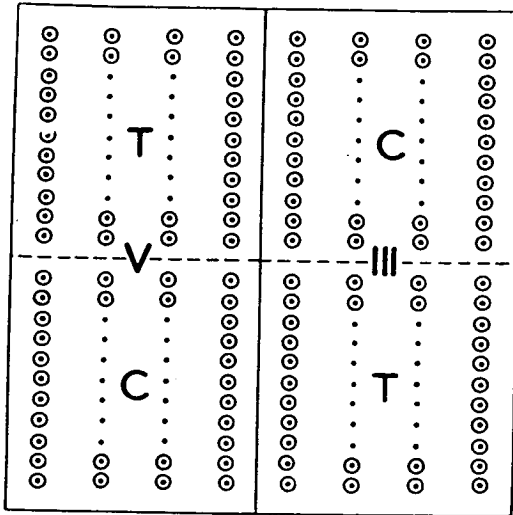
splashing during the summer.

Lay-out of site.

The site was divided into twelve blocks, each of eight rows of about eleven bushes (Text-fig. 5). One row on each side of each plot was regarded as a guard row, so that two such rows separated the rows that were to be assessed in adjoining plots. Six branches on each of four bushes selected at random from the two middle rows in each block were marked and used in the assessment. The percentage of infected leaves, estimated from thirty leaves on each selected branch, was calculated on July 9th and 29th, August 19th and September 15th. The results are given in Table 3.

Method.

The plots to be treated were dug by hand in March, and straw laid six inches deep between the bushes and between the rows at a rate of thirteen tons per acre on April 7th. In treated plots the straw was laid as far as the stools on the guard rows. The control plots were given normal cultivation using a rotary cultivator in place of the tined cultivator usually employed. This was necessitated by the random arrangement of the plots and the inability of the tractor to travel over the straw without disturbing it, or to turn round anywhere but at either end of the rows.



• ASSESSED BUSHES
 ○ UNASSESSED BUSHES
 Scale in feet 10

DETAILED PLAN OF BLOCKS III & V.

	C XII T	C XI T		
T	C	C	C	C
X	VIII	VI	IV	II
C	T	T	T	T
C	T	T	C	T
IX	VII	V	III	I
T	C	C	T	C

T TREATED PLOTS
 C CONTROL PLOTS
 Scale in feet 30

GENERAL PLAN OF SITE.

TEXT-FIGURE 5.

Effect of mulching on rate of spread of disease,
 1954.

Results.

Table 3.

Blocks.	<u>PERCENTAGE INFECTION</u>								Number of leaves counted	
	9.7.54 Treat. Con.		29.7.54 Treat. Con.		19.8.54 Treat. Con.		15.9.54 Treat. Con.		15.9.54 Treat. Con.	
I	15.9	17.2	88.4	81.0	98.4	99.7	100.0	100.0	27	28
II	9.9	1.2	91.1	74.5	98.2	97.3	100.0	100.0	13	9
III	7.2	5.5	78.7	59.4	99.6	99.3	100.0	100.0	33	22
IV	1.6	0.0	50.9	35.1	97.8	89.2	100.0	98.0	15	101
V	1.8	0.0	26.7	21.3	85.5	90.8	100.0	100.0	121	121
VI	0.0	1.4	9.1	27.2	53.7	89.4	99.5	99.1	196	107
VII	0.0	0.1	17.9	30.8	79.3	75.6	93.4	100.0	273	185
VIII	0.0	0.3	9.7	1.9	54.9	59.0	99.6	100.0	264	228
IX	1.0	0.2	10.0	21.1	74.1	87.7	100.0	98.7	409	155
X	0.0	0.0	7.0	33.9	51.5	89.0	99.3	100.0	304	82
XI	4.0	0.0	12.9	4.9	65.3	32.8	100.0	100.0	199	219
XII	1.3	0.0	9.0	5.7	53.4	45.5	97.5	99.1	237	342
Total	42.7	25.9	411.4	396.8	911.7	955.3	1189.3	1194.9	2091	1599
Average									174	133
Corrected means	1.9	0.7	32.2	30.3	91.3	94.1				

Since the number of leaves counted in constituent blocks was not the same, the percentages given in Table 3 were transformed before analysis, so that measurements were given equal precision throughout the assessment scale. The corrected means are given at the foot of the table.

A trend of more rapid spread of the disease on control than on treated bushes was discernible. On July 9th the difference was quite large, although not significantly so, the mean percentage infection on treated bushes being greater than that on control bushes. By July 29th this difference had diminished to two per cent, and on August 19th the mean for the control bushes was the greater.

No transformation was made of the figures for September 15th since infection was almost one hundred per cent throughout and the number of leaves required was, in many cases, no longer on the bushes. Analysis of the numbers of leaves counted on this date showed that significant differences existed between blocks, and that there was some difference in the level of defoliation between the treated and the control bushes. The treated bushes retained considerably more leaves than the control bushes at this time.

The results of the two field experiments suggest that both the method of cultivation, and the elimination of the overwintered leaves hold promise as additional control measures for this disease.

Fungicide tests on overwintered leaves.

Leaves collected in the autumn were treated with a number of fungicidal solutions during the winter, and their effect on the development of apothecia in the spring assessed.

Method.

During November 1953 large numbers of fallen leaves were collected from the Baldwins and placed in the open in the trays described above. On January 26th the boxes were emptied in the laboratory and the complete leaves picked out. Twenty-five leaves were dipped in each solution of each fungicide and divided between

two of the peripheral compartments of a tray. Each tray contained leaves which had received treatment with only one fungicide, at four concentrations, and thirty untreated leaves in the central compartment as control.

The leaves were allowed to dry for two hours before being placed outside for a further two days under cover. The trays were then exposed to the weather until the end of May when the effect of the treatment was assessed. On May 19th and 20th the trays were placed in the laboratory, the dry leaves immersed in water for three minutes and the surplus water shaken off. The leaves were then spread out to dry for five minutes before selected discs were removed and inverted over 1.7% agar in Petri dishes. Two discs one centimetre in diameter were cut from leaves treated with each concentration of fungicide, and two from the control leaves. After forty-eight hours the density of the discharge was assessed by counting the spores visible within the field of the microscope in the area in which the density was greatest. Taking one hundred spores in the field as the highest density, the figures were graded from one to four, allowance also being made for the size of the area of greatest density. This method was also used to record the discharge of other fungi, and notes were made on the appearance of apothecia and acervuli.

On May 24th the leaves were again placed in the laboratory, and being thoroughly wet, were allowed to dry for some time. Three discs were cut from each set of leaves and inverted over agar as before. Assessment was again made after forty-eight hours. In each case also, another assessment was made after a further forty-eight hours, but no change in the original assessment was recorded.

The totals for each treatment are given in Table 4.

Materials.

Five fungicides were used, each at four concentrations.

- (a) Sodium salt of dinitro-cresol as 0.8%, 0.4%, 0.2% and 0.1% aqueous solutions.
- (b) Sodium pentachlorophenate as 0.8%, 0.4%, 0.2% and 0.1% aqueous solutions.
- (c) Phenyl mercury chloride as 0.4%, 0.2%, 0.1% and 0.05% aqueous solutions. (The phenyl mercury chloride was ground up with equal proportions of Kaolin to form a wettable powder before being made up into solution).
- (d) Triethyl tin hydroxide as 0.4%, 0.2%, 0.1% and 0.05% aqueous solutions.
- (e) Cetyl pyridinium bromide as 0.4%, 0.2%, 0.1% and 0.05% aqueous solutions.

Results.

Table 4.

Density of spore discharge by *P. ribis* from treated leaves.

Treatment	Percentage Concentration					
	0.8	0.4	0.2	0.1	0.05	Control
Dinitro-cresol	0	0	0	0		28
Sodium pentachlorophenate	0	0	0	0		22
Phenyl mercury chloride		0	0	0	0	31
Triethyl tin hydroxide		0	0	1	9	36
Cetyl pyridinium bromide		2	1	11	17	24

The results given in Table 4 indicated that the fungicides tested, except cetyl pyridinium bromide, were effective at most concentrations. However, a study of the surfaces of the treated leaves, and of spores discharged from the discs by other species of fungus, showed that the effectiveness was largely confined to P.ribis, or to its perfect stage. On leaves dipped in 0.05% phenyl mercury chloride, for example, a number of acervuli containing malformed conidia were observed. Apothecia were absent even at the lowest concentration, and with increased concentration the eradication of the lesions became more pronounced, the area being marked by white spots due to the presence of empty spaces below the epidermis.

Sodium pentachlorophenate was entirely effective at every concentration, not only in preventing sporulation, but in killing the fungus in the tissues of the leaf.

Apothecia developed freely on leaves dipped in 0.1% and 0.05% cetyl pyridinium bromide, but at 0.4% they were very scarce, although the fungus in the lesions was not killed.

Triethyl tin hydroxide was effective against the formation of apothecia but not conidia at concentrations above 0.1%, and killed the hyphae within the lesions at 0.4%.

Dinitro-cresol was completely effective in suppressing sporulation and the number of empty lesions was very large even at the lowest concentration.

The figures for the assessments of the densities of all other fungal spores are given in Table 5. Since these assessments were for comparative purposes no identifications were made of the other species.

Table 5.

Density of spore discharge by fungi other than *P. ribis* from treated leaves.

Treatment.	Percentage Concentration.					Control
	0.8	0.4	0.2	0.1	0.05	
Dinitro-cresol	14	13	14	15		20
Sodium-pentachloro-phenate	6	14	14	13		21
Phenyl mercury chloride		0	5	5	10	18
Triethyl tin hydroxide		9	13	18	16	20
Cetyl pyridinium bromide		16	17	20	18	20

Indications from these results were that dinitro-cresol, sodium pentachlorophenate and phenyl mercury chloride were all effective in preventing ascospore discharge by *P. ribis* at the concentrations used. Phenyl mercury chloride, however, while being the most effective general fungicide appeared to be less active than the two sodium salts tested in preventing the formation of acervuli of *P. ribis*. It has been suggested elsewhere (17) that phenyl mercury chloride, being insoluble in water, may become fixed in the outer tissues of the treated leaf and continue to inhibit sporulation at the surface of the leaf for a considerable time. Such an effect might explain the continued development of fructifications in leaves treated with this material, combined with an eventual efficacy in inhibiting ascospore discharge. The

sodium salts tested, being readily water-soluble, possibly penetrated the leaf tissues at the time of application, halting the development of fructifications at an early stage.

DISCUSSION.

The development of modern agricultural machinery has resulted in wider spacing of the rows of black currant bushes, so that cultivation may be done automatically. The old methods of horse-drawn implements and manual labour have been supplanted by a quicker but less flexible type of cultivation, and although the older methods offered the bushes no protection against Leaf Spot, the situation has not been much improved. Nevertheless, the advances that have been made in relevant fields of research, allow a much more comprehensive approach to the problem of preventing diseases than was possible before. The correction of nutritional deficiencies and the breeding of new varieties, as well as improved methods of cultivation, have been shown to have some effect on the degree of susceptibility of the black currant to Leaf Spot.

The improvements in machinery have been followed by an improvement in the automatic application of protective sprays, so that any increase in diseases due to shortcomings inherent in automatic cultivation can largely be made good by the efficient application of spray materials. The choice of spray materials has been shown to be of vital importance when used on fruit, since the majority of fungicides leave residues containing traces of objectionable substances.

It has been mentioned that Wallace found that the black currant bush has a high nitrogen requirement, and that deficiency resulted in increased severity of Leaf Spot (14). Heavy pruning was also shown to have the same effect (4), while Roach (9) has recently

reported that the application of two per/cent urea as a spray reduces the level of the disease. These three methods rely on the principle that shifting the carbohydrate/nitrogen ratio in favour of nitrogen induces vigorous vegetative growth, and in the case of Baldwins in particular, which are weak growers and heavy croppers, the bush suffers less exhaustion through fruiting, and the susceptibility to Leaf Spot is therefore lessened.

The best possible conditions for the growth of the bushes can not reduce the severity of this disease to a level where it is no longer unnecessary to use some other method of control. It has already been pointed out that in the past research into the problem of control has centred round the application of sprays during the fruiting season.

The possibility has been investigated here of controlling the disease by the use of fungicides applied to the dead leaves on the soil. At this stage in the life-history of the fungus, the majority of fungicides can be safely applied at concentrations toxic to the fungus but not to the dormant buds of the black currant bush. That this approach has not been made before is almost certainly due to the fact that the perfect stage of P. ribis has only once before been reported in this country (16), and was reported by Blodgett (2) to be rare in the U.S.A.

The discovery of the perfect stage at Long Ashton in Somerset in the winter of 1952-53, showed not only that this stage could occur abundantly in this country, but also that it was probably widespread and the most important method of perennation of the

fungus. The suggestion that the primary infection resulted mainly from infections by conidia overwintered in the dormant buds could not be confirmed. Ewert (6) found that although overwintered conidia would germinate in the spring, no infection resulted. Attempts to induce overwintered conidia kept in vitro to infect leaves in the spring proved unsuccessful, but conidia formed in acervuli amongst the apothecia on overwintered leaves, were found to produce typical symptoms of Leaf Spot.

In the spring, therefore, two types of spore are available for primary infections, namely ascospores and conidia produced in the spring. If all the dead leaves could be removed and destroyed the amount of inoculum left would be very small indeed, but this would not be practicable in a plantation of any size. The field experiment carried out at Long Ashton in 1953 showed that the removal of the dead leaves was an effective method of reducing the amount of inoculum in the spring. Where this was supplemented by a ground spray during the winter the effect was increased, and the application of another spray to the leaves at an appropriate time in the spring further reduced the level of the primary infection.

It was found in 1954 that by using methods of cultivation differing from the normal, the spread of Leaf Spot could be retarded to some extent. However, the presence of an active inoculum in the spring was again shown to be the factor having the greatest influence on the severity of the disease. In the absence of fungicides during the months of June-August, the disease, once established, gathers momentum very rapidly and can practically defoliate a bush in

about two months. By removing or killing the inoculum the outbreak of Leaf Spot may be so delayed, and the centres of infection so scattered, that by the time the fruit is picked the disease has only just begun to appear. The application of a fungicide at this time has been shown to prevent the further spread of the disease until the leaves fall in late autumn (8), and the presence of residues is then of no importance.

Tests of fungicides have shown that several give complete suppression of all sporulation and also possess eradicant properties. An ability to kill Pseudopeziza ribis while having little effect on other fungi was observed with dinitro-cresol, which inhibited the formation of the perfect stage and also the formation of malformed conidia at low concentrations. This fungicide was used as a ground spray in February 1953 and proved effective in delaying the first appearance of the disease.

The use of a fungicide during the winter might not of itself be sufficient to give complete control, and it would then need to be supplemented by the application of a fungicide in the spring. The time of application is of the utmost importance if the fungicide is to achieve its maximum effect, and the more the level of primary infection is reduced the lower will be the level of infection at the time of cropping. Marsh and Maynard (8) found that the application of lime sulphur on March 30th gave no control of Leaf Spot, although in 1953 it had a definite effect when applied on April 8th. The nearer the time of application to the beginning of spore discharge, the greater the effect, but the time of discharge is dependent on temperature. It is suggested here that the apothecia mature readily when maximum temperatures

exceed 60°F. In the field experiments described above, the first assessments of percentage infection of the leaves were made on June 3rd 1953 and July 9th 1954. By July 1st 1953 the temperature had exceeded 60°F for a period of one hundred and eighty hours, whereas in 1954 the period was only one hundred hours. The temperature requirements are not known, but Blodgett (2) stated that the ascospores of P. ribis matured at temperatures of 54°F to 61°F, and discharged at temperatures between 61° and 75°F. Wilson (15) found with perithecia of Venturia inaequalis that as development proceeded the thermal requirements changed, and if the maximum requirements of the asci had not been met when a rise in temperature occurred, their maturation was extremely rapid.

It was found in the experiments on ascospore discharge that ^{the} two days after temperature rose to 58°F on March 28th 1953, mature ascospores were discharged after six hours in the moist chamber. When temperatures dropped a few days later to 48°-55°F. discharge occurred only after fifty-four hours in the moist chamber, and after a frost the time required was seventy-two hours. A week later, with temperatures of 50°-68°F, leaves required only a few minutes in the moist chamber for spore discharge to be initiated.

The temperatures of 61°-75°F necessary for the discharge of ascospores in the U.S.A.(2) were those required by apothecia developed on leaves of red currant, but a similar requirement exists in this country for those developed on the leaves of the black currant. The time of correct application of a fungicide depends.

ultimately on the thermal requirements of the maturing ascospores. A record of the temperature would enable a forecast of the beginning of discharge to be made, and the spray to be used with maximum effect.

In the course of this study it has been shown that lesions resulting from infection by ascospores and conidia grow very slowly and that the damage is caused by the immense number of lesions that develop in a small area of the leaf surface. Once the primary infections have taken place, the gathering momentum of the secondary infections is due to the summer rainstorms which carry the disease distally along the branches, from the upper surface of one leaf to the lower surfaces of the next above. Raindrops striking the upper surface of a leaf dissolve the mucilaginous matrix and splash the conidia to the lower surfaces of other leaves where conditions are most favourable to germination. Within two weeks conidia have been freed to infect other areas, so that leaves are rapidly killed and fall after about six weeks. The limited growth of the lesions is of considerable value since the application of a fungicide at any time will prevent further spread of the disease and enable the uninfected areas of the leaves to build up the reserves of the host.

The observations and experiments described here have been essentially of a practical nature, there being little information available on the disease in this country. It now seems probable that this disease can be effectively controlled by the application of fungicides during the winter, spring and autumn. If the amount of Leaf Spot can be so reduced in this way that the

application of sprays during the fruiting season is no longer desirable, then the difficult problem of residues need no longer be important.

SUMMARY.

1. The perfect stage of Pseudopeziza ribis was found in abundance on overwintered leaves of black currant at Long Ashton in Somerset. Specimens were lodged with the Commonwealth Mycological Institute at the request of the Director.
2. Experiments showed that the development of apothecia could be hastened by placing dead leaves in a moist chamber at 72°F. With regular collection of dead leaves as the year advanced, the time required for the asci and ascospores to mature was found to diminish. The first apothecia developed at the end of January after more than two weeks at 72°F, discharge first occurred at the end of March after six hours at 72°F, and germination first took place at the end of April after ten minutes at 72°F.
Acervuli formed on the lower surfaces of overwintered leaves liberated conidia also able to cause infections.
3. Natural discharge occurred in the field during the latter half of May 1953, and the first lesions appeared in early June. Although mature ascospores were readily discharged at the end of May 1954, the first lesions did not appear until early July. Comparison of the number of hours during which the temperature

was above 60°F showed that by mid-June 1953 there had been almost twice as many as by mid-June 1954.

4. Laboratory experiments on infection with ascospores and conidia were only successful when the spores were on the lower surfaces of the leaves. Ascospores were forcibly discharged for more than four millimetres, while conidia required the solvent action of water to release them from the mucilaginous matrix. In the absence of surface moisture, no increase in the number of lesions occurred. The growth of lesions was very slow once acervuli were formed and the maximum size recorded was 16 x 5.5 millimetres after five months. No difference in susceptibility to infection between young and old leaves was apparent.
5. The angular shape characteristic of the lesions was due to the barrier effect of the vein network of the black currant leaf. Penetration was directly through the cuticle and the wall of the epidermal cell following the formation of an appressorium. Both conidia and ascospores themselves acted as appressoria. The lesions became visible after seven to nine days and acervuli formed soon afterwards.
6. Secondary infection was due to raindrops carrying conidia to uninfected areas of the leaves and resulted in the disease spreading distally along the branches from leaf to leaf. Defoliation was caused by the rapid death of leaves on which immense numbers of lesions appeared simultaneously as a result of summer rainstorms.
7. In field experiments, removal of the dead leaves reduced the

level of primary infection and delayed the spread of the disease. A similar effect was achieved by the application of a fungicide a short time before spore discharge occurred.

8. The type of cultivation used during the summer had a retarding effect on the spread of the disease in the field, once the primary infection had occurred.
9. Fungicides were tested on overwintered leaves for their effect on sporulation in the spring. Phenyl mercury chloride was the most effective general fungicide at concentrations above 0.1% and dinitro-cresol and sodium pentachlorophenate were effective against P. ribis above 0.1%, other fungi being largely unaffected. The eradicant properties of dinitro-cresol were slightly greater than those of sodium pentachlorophenate.
10. It was concluded that the application of a fungicide to the dead leaves during the winter was^a valuable addition to the established methods of control. The use of a spray at the correct time in the spring, together with a post-cropping spray, would further reduce the incidence of the disease. A level might thus be reached where the use of sprays during the fruiting season would no longer be necessary and the problem of residues would no longer exist.

REFERENCES.

1. ADAM, W.B., DOWSON, W.M. and MARSH, R.W. (1948).
The effect of spray residues on canned black currants.
II. Long Ashton Res. Sta. Ann. Rep. for 1947, 163.
2. BLODGETT, E.C. (1936). The anthracnose of currant and gooseberry caused by Pseudopeziza ribis.
Phytopathology, 26, 115.
3. BOULD, C., HOBBS, E.W., KEARNS, H.G.H. and MARSH, R.W. (1953).
Some aspects of black currant culture.
Science and Fruit, J.W. Arrowsmith Ltd., Bristol, 120.
4. BRITON-JONES, H.R. (1926). A note on the leaf spot disease of black currants.
Long Ashton Ann. Rep. for 1925, 105.
5. CLINTON, G.F. (1914). Notes on plant diseases in Connecticut.
Rep. Conn. Agr. Exp. Sta. 38, 12.
6. EBERT, R. (1910). Die Überwinterung von Sommerkonidien pathogener Ascomyceten und die Widerstandsfähigkeit derselben gegen Kälte. Zeitschr. Pflanzenkr., 20, 129.
7. MARSH, R.W. and DICKINSON, D. (1945). The control of black currant leaf spot by dithiocarbamate sprays and the effects of spray residues on the canned fruit.
Long Ashton Res. Sta. Ann. Rep. for 1944, 150.
8. MARSH, R.W. and MAYNARD, J.G. (1930). Black currant leaf spot disease and its control.
Jour. Min. Agric. 37, 255.
9. ROACH, F.A. (1953). Some observations on the occurrence and correction of nutrient deficiencies in fruit trees and bushes.
N.A.A.S. Quarterly Review, 20, 363.
10. STEWART, F.C. and EUSTACE, H.J. (1901). An epidemic of currant anthracnose.
New York (Geneva) Agr. Exp. Sta. Bull., 199.
11. SPINKS, G.T. (1948). Black currant breeding at Long Ashton.
Long Ashton Res. Sta. Ann. Rep. for 1947, 35.
12. SUIT, R.F. (1945). Currant leaf spot control.
New York St. Agr. Exp. Sta. Bull., 709.
13. WALLACE, T. (1925). Experiments on the manuring of fruit trees - II.
Jour Pomology, 5, 1.

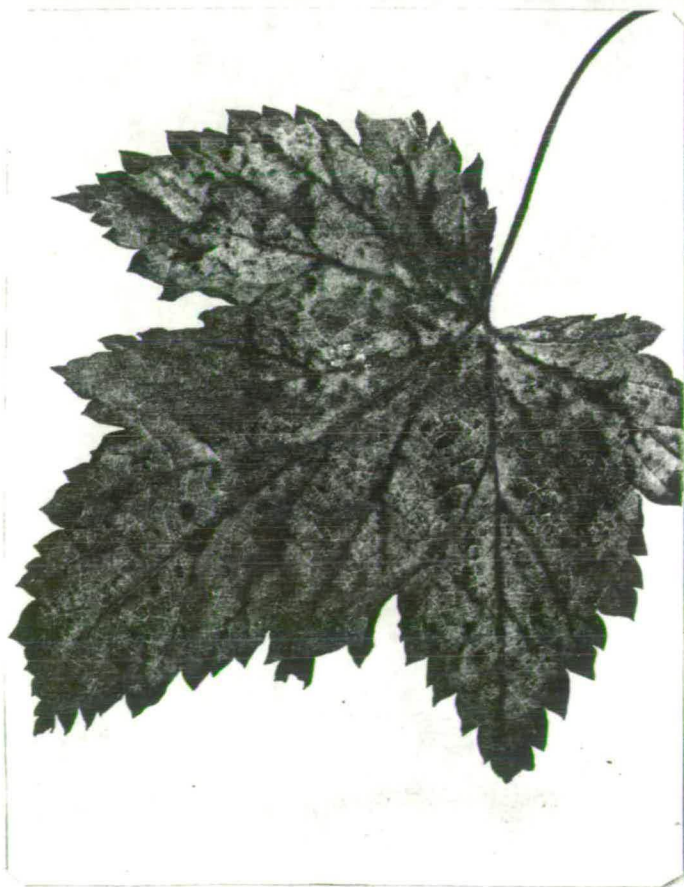
14. WALLACE, T. (1938). A field experiment on the manuring of black currants. Jour. Pomology, 16, 127.
15. WILSON, E.E. (1928). Studies of the Ascigerous Stage of Venturia inaequalis (Cke.) Wint. in relation to certain factors of the environment. Phytopathology, 18, 375.
16. WORMALD, H. (1946). Diseases of Fruits and Hops. Crosby Lockwood and Son Ltd., London, 173.
17. CORKE, A.T.K. (1955). Black currant leaf spot: II. Laboratory tests of fungicides for the prevention of sporing of Pseudopeziza ribis on overwintered leaves. J. hort. Sci., 30, 197.



1



2



3

PLATE II.

Fig. 4. Acervulus formed on an overwintered leaf of black currant showing mature conidia.

x 495.

Fig. 5. Mature apothecium on an overwintered leaf of black currant.

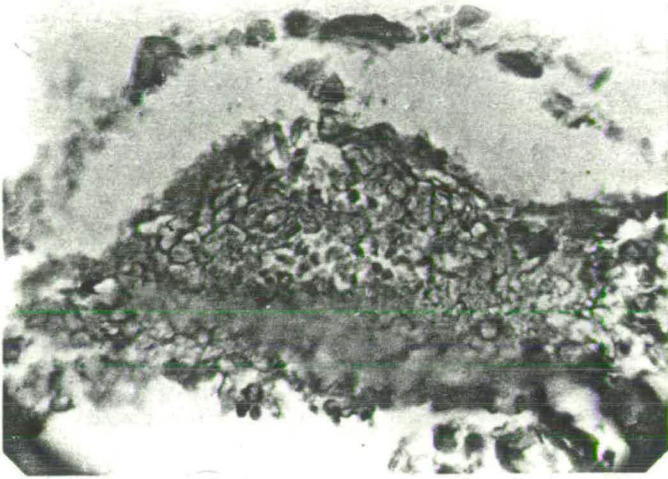
x 495.

Fig. 6. Mature asci and paraphyses.

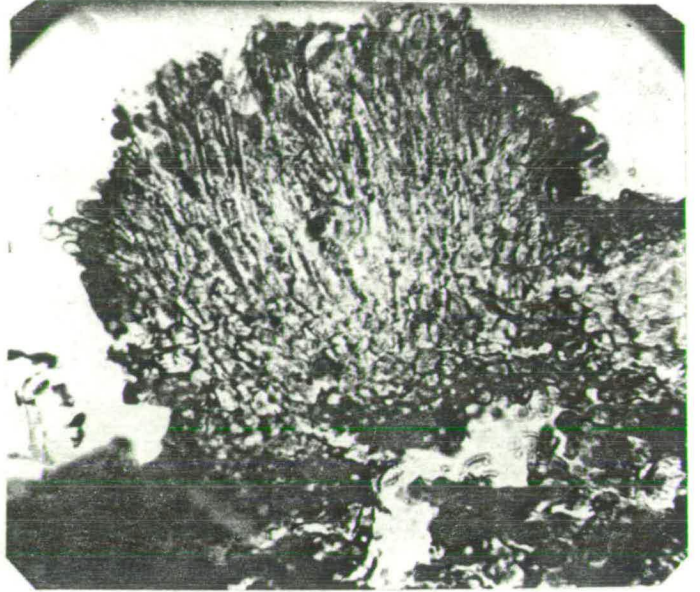
x 495.

Fig. 7. Germinated ascospore six days after discharge showing the appressorium and bi-lobed primary hypha.

x 2,600.



4



5



6



7

ACKNOWLEDGEMENTS.

The author wishes to acknowledge with gratitude the advice and help given during the course of this study by Prof. Sir. W. Wright Smith, and for permission to publish some of the material in the Annual Report of the Long Ashton Research Station for 1953, and the Journal of Horticultural Science, Vol. 30, No. 3, July 1955.

Grateful thanks are also due to Dr. M. Wilson and Dr. W. Blyth for help and criticism, to Mr. D. Henderson for collecting infected material from Dunkeld, and especially to Dr. E. Muller of Zürich for sending material and publications from Switzerland and making available his knowledge of the disease in Europe.

The author also wishes to thank the Director and Staff of the Long Ashton Research Station for facilities and help in the field work carried out at Long Ashton.

Thanks are particularly due to Mr. R.W. Marsh and Dr. R.J.W. Byrde for advice throughout the study, and to the Department of Mycology for assistance in assessing and recording experimental results; to Mrs. D. Fowles and Mr. G.M. Clarke for carrying out the statistical analyses; to Mr. G. Jones for reproduction of the Text-figures and the photographs on Plates IX - XIV (Part I) and Plate I (Part II), and Mr. G.E. Clothier, through whose kindness the meteorological records were made available.